INDUSTRIAL EXPOSURE PROJECT ON BEER PRODUCTION, QUALITY ASSUARANCE AND MANAGEMENT OF BREW SECTION

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

Submitted By
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June 2025

UNITED BREWERIES LIMITED

Monday, 31st March 2025

TO WHOM IT MAY CONCERN

This is to certify that Ms. Supriti Sanki, Daughter of Mr. Manoranjan Sanki, a Student of Msc Biotechnology at Sister Nivedita University underwent an Internship Training Course at this Brewery from 01.02.2025 to 31.03.2025. During this period, she has successfully completed the Training on "Industrial Exposure Project on Beer Production, Quality Assurance and Management of Brew Section".

She was found intelligent, sincere, and hardworking during her training at the Brewery, and we wish her every success in her future.

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CERTIFICATE

This is to certify that the dissertation of an industrial internship report entitled "Industrial project report on beer production, quality assurance and management of brew section" submitted by Supriti Sanki in partial fulfilment of the requirement for the degree of Master of Science in Biotechnology from Sister Nivedita University, Kolkata, West Bengal bearing the registration No.230020458125 and roll no. 2322210002045 is a Bonafide dissertation report carried out by Supriti Sanki under the guidance and supervision of

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "INDUSTRIAL EXPOSURE PROJECT ON BEER PRODUCTION, QUALITY ASSUARANCE AND MANAGEMENT OF BREW SECTION" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from February to April under the supervision of Mr. Ashok Basak Head Brewer of United Breweries Ltd, Kalyani.

I acknowledge that I have read and understood the UGC regulations, 2018 (*Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions*).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software-. https://smallseotools.com At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled " INDUSTRIAL EXPOSURE PROJECT ON BEER PRODUCTION, QUALITY ASSUARANCE AND MANAGEMENT OF BREW SECTION ", submitted by Ms. SUPRITI SANKI (Registration No. 230020458125 Enrolment No. 2322210002045) to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfillment of the requirements for the Master of Science degree.

Date: 03.06.2025 .

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I would like to take this opportunity to express my heartfelt gratitude to everyone who has contributed to making my dissertation project at **United Breweries Limited**, **Kalyani** a truly remarkable experience. It has been privilege and honour to work in such stimulating and intellectual environment.

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Abbreviation:

Abbreviation	Meaning
FV	Fermentation vessel
BBT	Bright beer tank
PU	Pasteurization unit
CIP	Cleaning in process
DMS	Dimethyl sulphide
OPRP	Operation pre-requisite program
$KMNO_4$	Potassium Permanganate
RDF	Real degree of fermentation
MGF	Multi grade filter
MCF	Micro cartridge filter
ACF	Activated carbon filter
RO	Reverse osmosis
PPM	Parts per million
WTP	Water treatment plant
CO_2	Carbon dioxide
$C_6H_{12}O_6$	Glucose
C_2H_5OH	Ethanol

Abstract:

Beer is an alcohol beverage consumed throughout the world having an alcohol percentage of below 8% in India. Though beer is an alcohol beverage, still it has various health benefits including good for heart health, prevents kidney stone, good for teeth, skin and hair health.

My dissertation project work is based on the complete exposure of a beer industry: **United breweries Ltd, Kalyani, West Bengal**. My project work includes the production of beer from starch raw materials like rice, malt. This whole beer production involves malting, milling, mashing, extract separation, hop addition, boiling, cooling & aeration, fermentation, separation of yeast, aging and maturing the beer and the reduction of diacetyl (Off-flavour) from beer by yeast management system.

Starting the production from screening of raw materials, passes to milling, production of wort, (the sugary liquid which is being consumed by yeast and to produce alcohol) which involves passing from mash kettle, lauter tun, wort kettle, whirlpool; then passes to fermenter where fermentation is going to take place at a duration of 6-7 days, Fermentation is a process by which yeast converts the glucose in the wort to ethyl alcohol and carbon-dioxide also produces heat giving the beer both alcohol content and carbonation. After completion of yeast activity, the matured beer undergoes filtration in order to remove any solid particles. Finally, the beer is produced.

The Off-flavour(diacetyl) of beer is generated by abnormal yeast management, lactobacillus, which spoils the beer is also a causing bacterium for the off-flavour. If diacetyl is present in the beer, then a butterscotch flavour is generated. It depends upon the temperature, yeast management etc. This diacetyl is reduced by the proper yeast management. Yeast growth is maintained during beer production. Under anaerobic condition yeast produce alcohol. The number of yeast cells are calculated by using microscope.

Keywords: Fermentation, Brew section, Yeast management, Filtration, Alcohol, Maturation, off-flavour.

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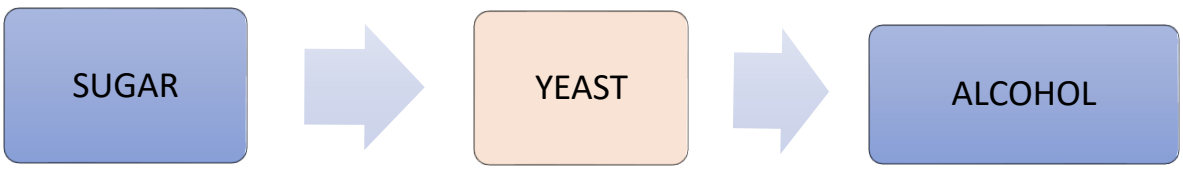
INTRODUCTION to Beer

Beer is an alcoholic libation produced by saccharification of starch and fermentation of resulting sugars. The starch and saccharification enzymes are derived from malted cereal grains, most commonly malted barley and malted wheat. Its flavour with hops, which add bitterness and act as a natural preservative, though other seasonings similar as herbs or fruits, may sometime be included. The preparation process of it termed as brewing.

Beer is the world's most extensively consumed potables beverages and is the third-most popular drink overall, after water and tea. The strength of the beer is generally around 4% to 8% alcohol by volume.

Beer is one of the world's oldest prepared beverages; possibly dating back to the early Neolithic or 9500 BC, when cereal was first farmed and is recorded in the written history of ancient Iraq and ancient Egypt. Beer was also being produced and vended by European monasteries. During the Industrial Revolution, the product of beer moved from artisanal manufacture to industrial manufacture and domestic manufacture ceased to be significant by the end of the 19th century. Today the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers ranging from brewpubs to regional breweries.

Chemistry of Beer :



The chemical compounds in beer give it a distinctive taste, smell and appearance. The majority of compounds in beer come from the metabolic activities of plants and yeast and so are covered by the fields of biochemistry and organic chemistry. The main exception is that beer contains over 90% water and the mineral ions in the water (hardness) can have a significant effect upon the taste.

CO2 plays a major role in beer production. CO2 is very soluble in beer, and its solubility increases with pressure and decreases with temperature. The amount of CO2 dissolved in beer is most often referred to in terms of volumes. The gas gives a refreshing sensation for the beer. The carbonation is also responsible for the bubbles that create the foam which is important to retaining the beers temperature. The volume of CO2 inside the beer is related to the foam consistency. Different beer styles have distinct volumes of CO2 required. Volumes of CO2are defined as the volume the CO2 gas would occupy if it were removed from the beer at atmospheric pressure and 0°C, compared to the original volume of beer. Thus, if a quart of beer were carbonated to 2.5 volumes and all the CO2 were removed from the beer, it would occupy 2.5 quart.

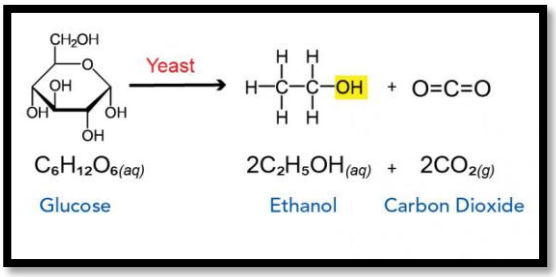


Figure 2: Production of Ethanol by Fermentation

Raw Material:

Requirements for Production

1. Malt
2. Adjunct
3. Water
4. Yeast
5. Miscellaneous additives

MALT:

Malt is barley or other cereals like wheat which has been germinated under controlled conditions followed by draying to terminate the germination before an actual new plant has developed and this process known as malting. The house where this process is going on is termed as Malt house. The aim of the malting is to loosen up the endosperm by degrading the endosperm cell walls and produce enzymes for further degradation of the content of the endosperm cells during mashing.

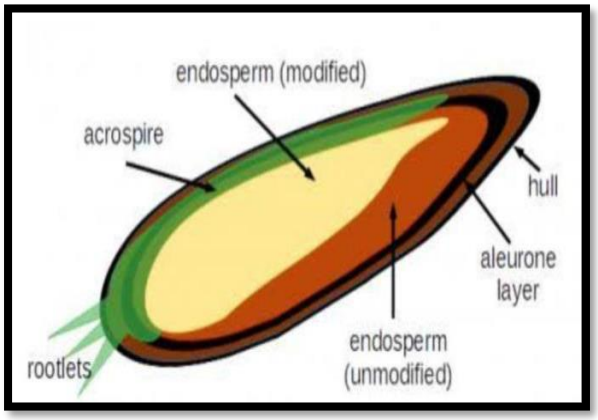


Figure 3: Cross Section of Malt

Figure 4: Malt

Barley is the principal grain used in brewing for a number of reasons:

- The plant can be grown in many parts of the world ranging in latitude from near the polar circles to the equator.
- Its uniform and convenient size makes it easy to handle on an industrial scale.
- The grain contains 60 – 65% by dry weight of starch. Together with proteins, enzymes, vitamins and minerals, the grain provides a complete package for yeast nutrition.
- It contains sufficient lipids for yeast metabolism under anaerobic conditions, in some other grains excessive levels of lipids can have deleterious effects on beer processing or quality.
- The husk is relatively tough and can form a filter bed in the brewhouse.

The malting process is 3 types:

Steeping: In this process the germination is initiated by increasing the moisture content of the grains from about 12% to about 45%. Barley is soaked in water to simulate the conditions that start germination or growth. This is carried out in a steep tank. During steeping the grain is aerated, (normally by draining and drawing air through the wet grain bed, before resoaking) to reduce the numbers of grains dying off due to "drowning" and to increase the rate of water uptake. The barley is usually steeped and aerated a number of times – at the end of steeping the moisture content of the barley should be around 45%, dependant on the type of malt being produced.

Germination: In this process the grains are allowed to germinate for 3-7 days depending on the vitality of the grains. Along with the growth of the roots, the endosperm is modified, and active enzymes are built up. On completion of steeping, the barley seed is allowed to grow. During germination two major changes occur:

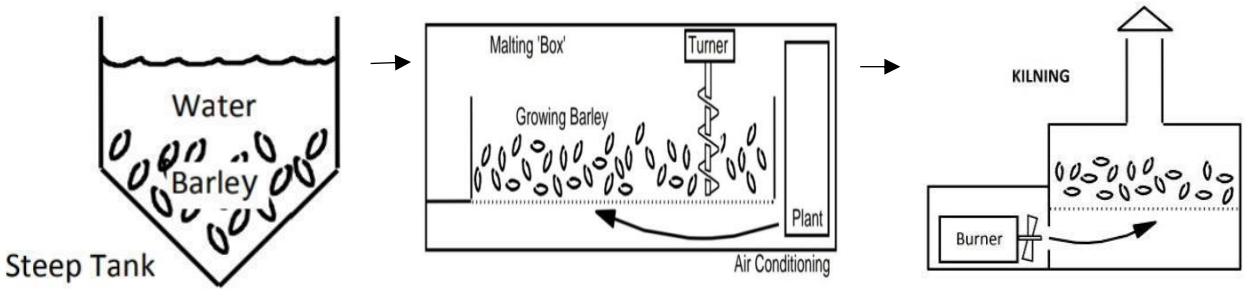
Firstly, hormones stimulate the production of enzymes in the aleurone layer.

Secondly, these enzymes start to act. During malting they will break down the gummy cell walls and break down the protein matrix inside the starch containing cells. This breakdown releases the starch granules making them accessible for conversion into sugar. The changes taking place during germination are called 'modification'. The maltster can influence the degree of modification during malting by controlling the moisture content of the grain, its temperature and the time allowed for germination.

Kilning: In this process the grains are gently dried down in order to preserve the activity of the enzymes and to get a stable product with the right flavour.

During this stage of the malting process, water is removed from the green malt. The malt then becomes stable and can be stored without deterioration. The malt may also be slightly roasted to give it colour and flavour. The malt kilning process is manipulated so that the malt is dried at a relatively low temperature (around 50°C) using high flows of air. When the malt is dry, with a moisture content of around 10%, the kilning temperature is increased (up to 90°C) so that the malt develops colour and flavour. At the completion of kilning, the malt's moisture content will be 3 – 5%. The finished malt is normally allowed to rest for at least two weeks before use as this produces a more consistent final product for the brewer to use.

Malting Process Flow Diagram:



What does malt contain?

Like most commodities, malt is taken for granted. However, the myriads of components it provides us with is remarkable. Important chemicals contributed to the brewing process by pale malt include starch, protein, amino acids and peptides, phosphate, polyphenols, melanoidins, O- and N-heterocyclic compounds, lipids and sterols, beta-glucan, vitamins, metal ions, and enzymes. In addition, malt husk material provides the filter medium for mash tun and lauter tun separation systems.

Starch: The starch granules comminute from the malt make up the greatest portion of the extract. This cereal starch is in granular form with two forms: Amylopectin (70-80%) & Amylose (20-30%).

Malt Adjunct: Adjuncts are un malted grains (such as corn, rice, rye, oats, barley and wheat) used in brewing beer which supplement the main mash ingredient, often with the intention of cutting costs, but sometimes to create an additional feature, such as better foam retention, flavours or nutritional value or additives.

Water: Beer consists of more than 92% water. Sourced water is treated to brewing quality standards. The composition of the water that is to be used in brewing is very important. The quantity and character of dissolved salts are much significant while pH is secondary interest. Water containing calcium and magnesium sulphate with a permanent hardness of 200-300 ppm is usually stable. The characteristics of good water for brewing are a pH of 6.5-7.0 less than 100 ppm of calcium and magnesium carbonates, 250-500 ppm calcium sulphate, 200-300 ppm sodium chloride and less than 1 ppm iron.

Yeast: During fermentation, yeast cells convert cereal-derived sugars into ethanol and CO2. At the same time, hundreds of secondary metabolites that influence the aroma and taste of beer are produced. Variation in these metabolites across different yeast strains is what allows yeast to so uniquely influence beer flavour. (Saccharomyces cerevisiae) yeast is used in the production of beer in kingfisher.

Figure 5: Pure culture of yeast

MISCELLANEOUS ADDITIVES:

Gypsum **–** Used to increase the hardness of water, it reacts with phosphate from malt and reduces the pH toward the optimum for mashing. Calcium ion also stabilized many enzymes.

✓ ***Calcium Chloride*** **dihydrate–** Used to increase the hardness of water.

✓ ***CaSO4 dihydrate-*** pH control and yeast nutrition.

✓ **Neutrase:** As a protease enzyme to break down proteins in wort

✓ ***Termamyl SC*****: –** Gelatinization of adjuncts.

✓**Lactic Acid:** pH adjustment for enzymatic activity.

✓ **Attenuzyme –** For RDF control.

✓ **Brewtan B:** For flavour stability, clarity, inhibit oxidation, trub separation.

✓ **Whirl flock –** Flocculating reagent is that is added during wort boiling and lead to flocculate and precipitate the unwanted protein of the wort.

✓ **Laminex Maxflow 4G:** Improves beer filtration & mash separation.

✓ **Potassium Meta Bisulphite –**Also active in limiting growth of wild yeasts & bacteria in beer

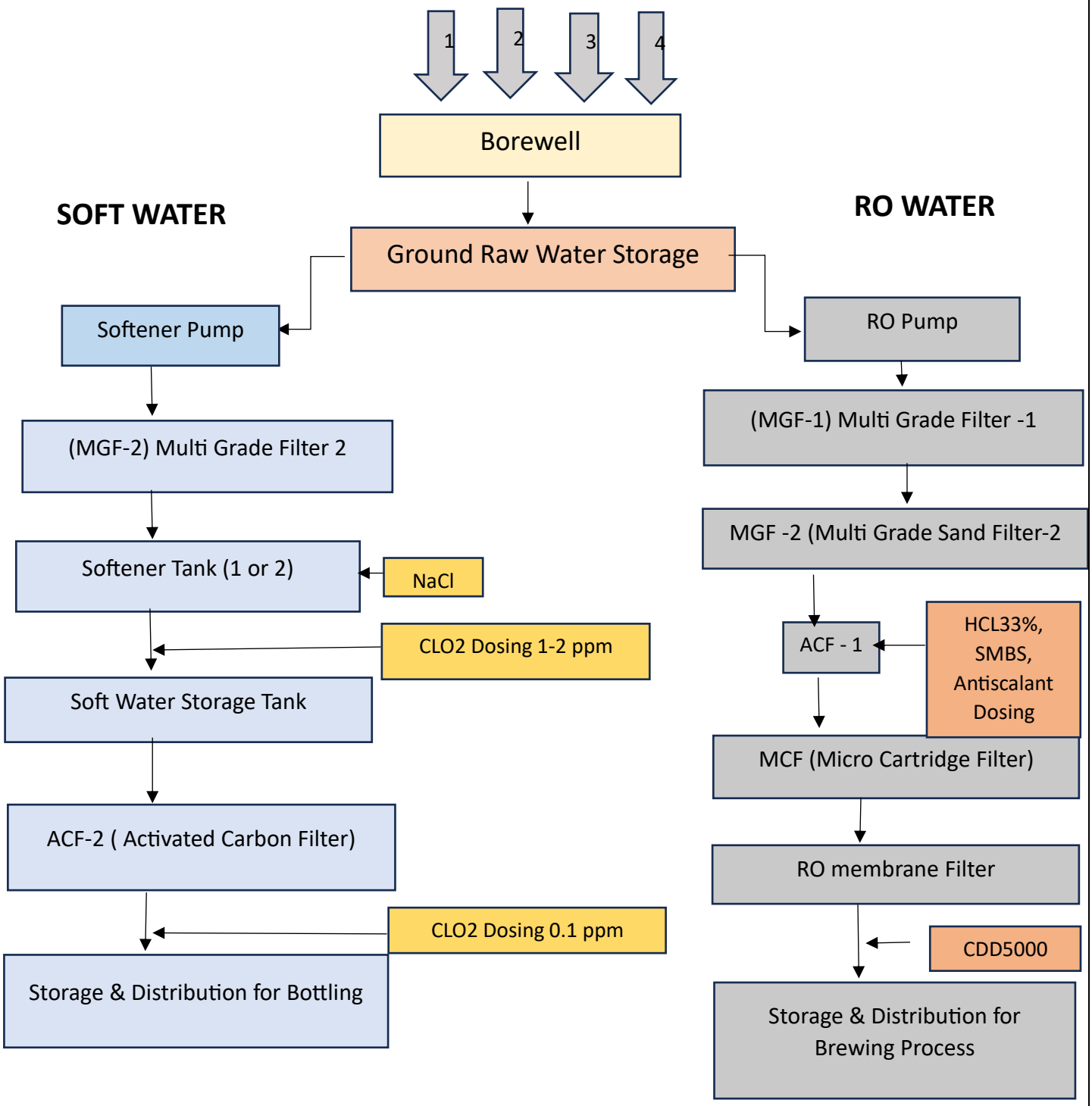
✓ **Caramel –** Their uses are optional and use to increase the desirable colour of the beer to the acceptable level being sought.

Hops: Hops were originally introduced by brewers to help inhibit the growth of beer spoiling bacteria, to maintain flavour stability, and to help retain the head (foam) of the beer. There are over 50 different varieties of hops which are grown commercially in Germany, southern England, southern Australia, Tasmania and Washington State.

Alpha acids and Beta acids are the resins contributing to bitterness. They are conventionally measured on a mass percent basis. Of the two, alpha acids are the most dominant in bitterness contribution. Both alpha and beta acids are not soluble in water, in order to allow them to contribute to the bitterness of the beer, they must be modified. The bitterness, flavour and aroma of hops are released into the wort through boiling. The timing of additions of hops to the wort is called a hopping schedule. The amount of bitterness imparted to the wort in called utilization. There are four basic classifications of hop use. Bittering, flavouring, finishing and dry hopping.

Sugar: Sugar is also an additional carbon substance to the yeast, and it is also a malt adjunct. It doesn't require any enzymatic activity because yeast can use directly. Approximately 80-90% of the weight of these malt adjuncts is extracted into the wort.

WATER TREATMENT PLANT



Water Testing & Required Parameters

Table 1

Serial No	Parameter	Specification	Unit
1	Raw water CLO2	0.8	Ppm
2	Ro water CLO2	>0.2	Ppm
3	Ro water hardness	>=60	Ppm
4	Soft water CLO2	>0.2	Ppm
5	Soft water hardness	<10	Ppm
6	RO 2 permeate	30	KL
7	RO – TDS permeate	40-50	Ppm
8	RO reject TDS	1100-1200	Ppm
9	RO – PH	~6.7	
10	Borewell water withdrawal	>1220	Kl/day
11	RO feed pump flow rate	40	M3/h

Brewing Process

MALT MILLING PROCESS:

The malt and adjunct are important from other supplier by local transport and store in Silo. There are Four silos of capacity100 MT (rice) & 100(malt silo)200 MT (Malt Silo),200 MT (Malt Silo). There is a blower provision to circulate the air inside the silo, to avoid the dampening effect of the malt. It passes from silo through different cleaning process**.**

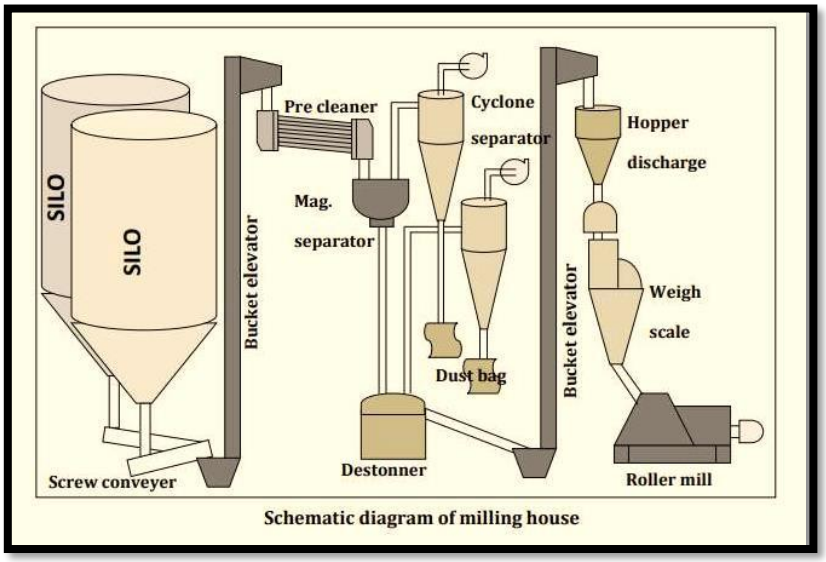


Figure 6: Schematic diagram of milling house

Roller malt milling:

In roller mill there are six rolls for milling process and gapes between rolls are respectively 1.4:0.9:0.65.

Brew House Operation

The brew house consists of the following unit:

- Adjunct kettle
- Mash kettle I, Mash kettle II
- Lauter Tun I, Lauter Tun II
- Holding Tank I, Holding tank II
- Wort kettle I, Wort kettle II
- Whirlpool

- **MASHING:**

ADJUNCTKETTLE

Gelatinization of broken rice is done here. It's a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen& oxygen) to engage more water. This irreversibly dissolves the starch granules. Penetration of water increases randomness in the general starch granule structure and decreases the number and size of crystalline regions. Crystalline regions don't allow water entry. Heat causes such regions to become diffuse, so that the chains begin to separate into an amorphous form. During gelatinization, water act as plasticizer.

Figure 7: Adjunct kettle

Enzymes used in Adjunct Kettle :

- **Termamyl SC 4X –** This chemical containsα-amylase enzyme which helps to break the alpha bonds inside the starch molecules.
- **Calcium Sulphate –**This chemical helps to maintain the pH of solution.
- **Gypsum**

MASH KETTLE

Ground malt and solid adjuncts (grist) are mixed with a set volume of water to achieve a specified temperature. The mash is then allowed to stand for a period of time, typically around

an hour, during which the enzymes in the malt convert the starch to sugars to produce a sugary liquid called wort. Depending on the type of malt, it may be necessary to heat the mash to specified temperatures so different enzymes work close to their optimum temperature. During mashing:

- Cell wall components may be broken down to release the starch (non-isothermal mashes only, where the endosperm has not been fully broken-down during malting)
- Proteins are broken down to amino acid.
- Starch is broken down and converted to sugars.
- The pH drops.

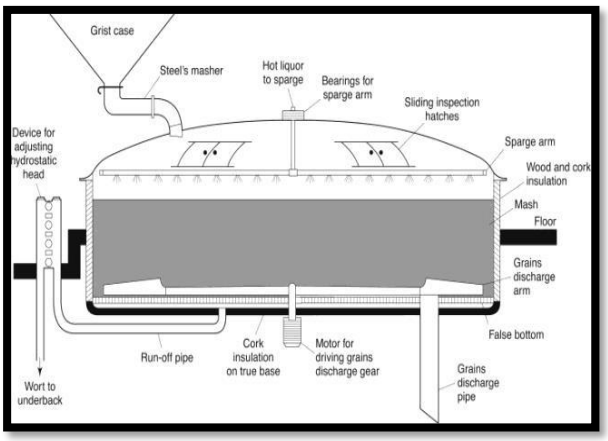


Figure 8: Diagram of Mash kettle

Figure 9: Mash kettle

Mashing is the process by which sweet wort is prepared. It involves mashing in, the mixing of the milled grist and water at the correct temperature and in the correct proportions to obtain the mash. After a period, with or without temperature changes, during which the necessary biochemical changes occur, the liquid sweet wort, which contains the extract, is separated from the residual solids, the spent grains. The purpose of mashing is to extract the soluble sugars, dextrins and inorganic substances from the malt grains in addition to converting the insoluble material (starch and protein) to a fermentable form. It's probably the most important process in the brew house. Here saccharification test is tested.

<u>Enzymes used in Mash Kettle</u>:

- Calcium chloride –This is used to correct the mineral deficiencies in water is to be used for brewing.
- Calcium sulphate – This chemical acts as a buffering agent to maintain the ph.

- Attenuzyme pro – This chemical contains glucoamylase. This helps in saccharification, catalysing reverse reaction in which the dextrose molecules get combined to form maltose and isomaltose. This chemical also helps in achieving high real degree of fermentation (RDF).
- Laminex – maxflow 4G, Neutrase (0.8) are also used in mash kettle and stored at refrigerator.

Table 2 ***Optimal rest temperature for major mashing enzymes:***

TEMPERATURE	ENZYMES	BREAKDOWN
45°C	**β-glucanase**	**β-glucans**
52°C	**Proteases**	**Proteins**
62°C	**β-amylase**	**Starch**
72°C	**α-amylase**	**Starch**

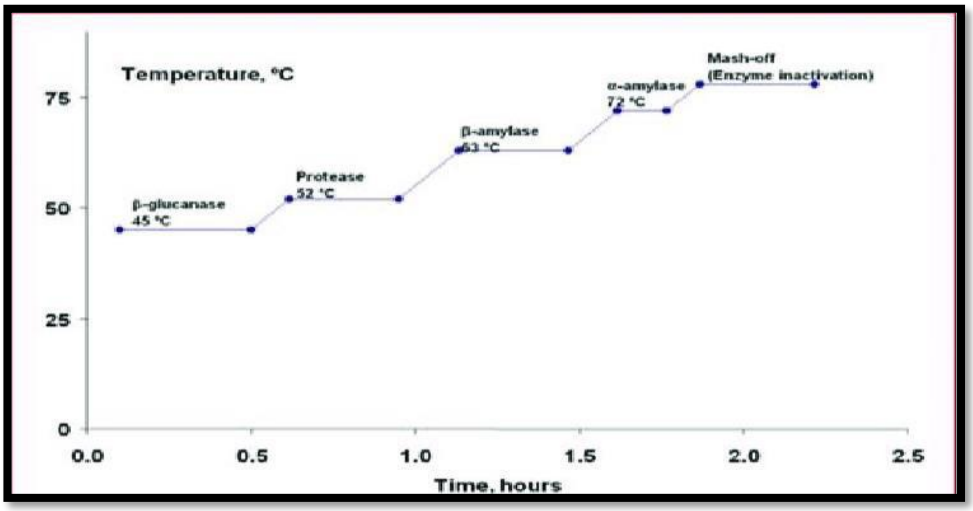


Figure 10: Graphical representation of Mashing

❖ Lautering

LAUTER TUN

It is a process in brewing beer in which the mash is separated into the clear liquid wort and the residual grain. 5 mins rest during lautering of mash for bed formation, lautering usually consists of 3 steps: mash out, recirculation, and sparging. Mash out is the term for raising the temperature of the mash to 76 - 78°C. This stops the enzymatic conversion of starches to fermentable sugars, and makes the mash and wort more fluid. Recirculation consists of drawing off wort from the bottom of the mash, and adding it to the top The mash itself functions much as a sand filter to capture mash debris and proteins. Sparging is forcing water through the grain to extract sugars from the grain. Here water is spreading two halves', one is 1st sparging using of 70-75 Hl water by 90-100Hl wort collected then 2nd sparging using of 30-35 Hl water, collected 50Hl wort. This is a delicate step; as the wrong temperature or pH will extract tannins

from the chuff (grain husks) as well, resulting in a bitter brew. Total process time of lautering is 140 mins.

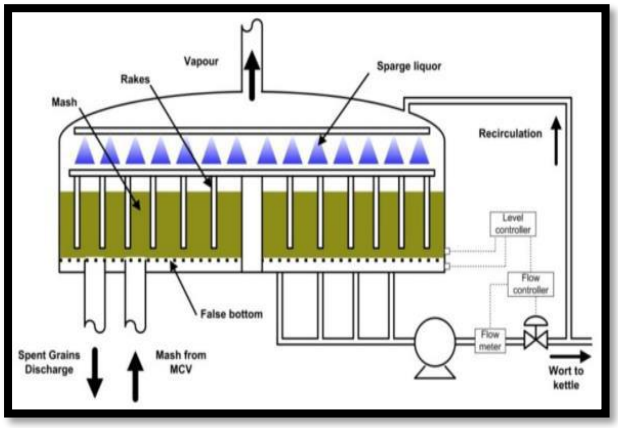


Figure 11: Schematic diagram of Lauter tun

Figure 12: Lauter Tun

Operation of Lauter Tun:

- After mashing from mash kettle rest for 10-15 min taken.
- Wort is circulated till the wort got clear, after the bed formation on the false bottom.
- Transfer the wort to the wort kettle.
- The gravity is checked.
- Then sparging of hot water done with temperature about 78°C.
- Final gravity is checked, and clear wort transferred to wort kettle.
- The remaining spent grain is removed to the grain kettle. Here near 1100kg spent grain collected per brew and used as cattle feed.

Wort clarity:

Wort separation also aims to achieve consistent wort quality. This can be difficult to measure but typical parameters would be: -

• Wort haze - should be < 50 EBC 10 minutes after the start of run off. This can be measured with an in-line haze meter.

• Suspended solids - no more than 10 to 15 ml as sediment after 2 hours stand in an Imhoff cone.

High hazes in wort can lead to a number of problems either in the brewhouse, or in the process downstream, including

• Run-off problems.

• Excessive volumes of trub / high hazes.

• Flavour problems.

• Fermentation problems (e.g. Due to smothered yeast in extreme circumstances)

• Variable yeast flocculation.

Spent Grains:

The waste husk after the wort has been removed is a valuable by-product because it can be utilised as animal food, typically for cattle. Where used as animal feed, it is necessary to maintain hygienic conditions for the storage and handling of the spent grains, and to ensure no contamination by foreign materials such as oils. Traceability is typically also required. In some breweries, the grain is used as biofuel. To be suitable for this it often has to be further dried using relatively high-pressure belt presses and dried further using the hot exhaust gases from the furnaces.

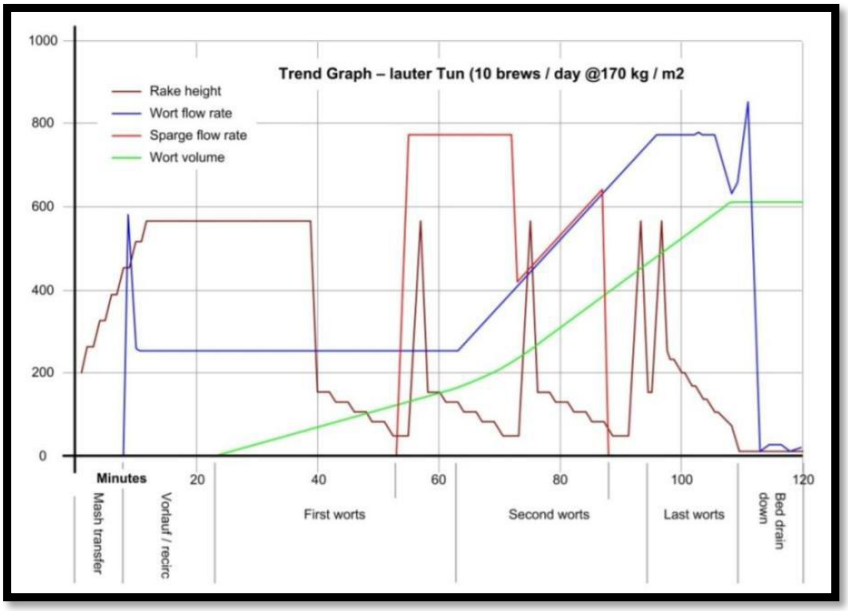


Figure 13 : Trend Graph of Lauter Tun

HOLDING:

HOLDING TANK

Holding tank is used to hold the wort extract. Temperature in this tank should be 78°C.

Figure14: Holding Tank

❖ WORT BOILING

Wort Kettle: Wort boiling may be regarded as the turning point in the brewing of beers, many complex reactions take place during wort boiling. As the wort is heated the residual amylases and other enzymes are inactivated which terminates the mashing process and fixes the carbohydrates composition of the wort. At the boiling point, the wort is sterilized, and the micro flora of the malt, hops and other adjuncts used are destroyed wort boiling is a key step in the production of quality beer.

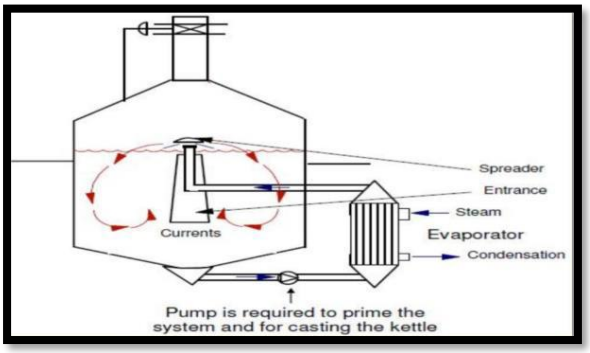


Figure 15: Boiling Process in Wort Kettle with External Wort Boil

Figure 16: Wort Kettle

When the wort has been separated from the malt husk, it is boiled. There are several reasons for doing this:

• To sterilise the wort. Brewing raw materials such as malt, hops and occasionally brewing water itself are infected by micro-organisms. These contaminants are washed into or added to the wort and remain viable, and therefore have to be killed during the brewing process to prevent wort and beer spoilage.

• To stabilise the wort. Above 50 – 80°C, enzyme structure is broken down and the enzymes lose their activity. Thus, a small quantity of enzymes that converted the starch into sugar and the protein into amino acid may not be fully denatured by the final higher temperature stages of mashing, and sparging. Some brewers add external enzymes, such as thermostable beta-glucanase or alpha amylase, intended to help with wort filtration or adjunct degradation (e.g. rice or maize). These enzymes are more heat stable and are active throughout mashing but will be de-activated during wort boiling. It is important that all enzymes are destroyed by boiling, otherwise they would continue working, which would change the profile of the beer.

• To dissolve the bittering resins from the hops and to stabilise them. Hops or hop extracts are added because the bitter resins (alpha acids) dissolve better in hot wort. These alpha acids need to be modified by 'isomerisation' reactions which are heat induced to stabilise the bitterness that is typical of beer flavour.

• To dissolve oils that contribute to hop aroma in the final product, though these generally only remain if the hops are added late, and the oils are not given time to be boiled off.

• To denature and coagulate some of the protein derived from the malt. Protein has the potential to make packaged beer go cloudy as it ages. Its removal at this stage will help protect the beer's stability.

• To develop wort colour and flavour through the action of heat on sugars and amino acids (the chemical reaction between sugars and amino acids is known as the Millard Reaction).

• To increase the strength or concentration of the wort. Wort concentration is a factor in ensuring that the chemical changes described above actually occur. It is also important in the production of strong beers whose original gravity is higher than that of the wort coming from the wort separation system. The amount of water removed during the boil is directly proportional to the rate of evaporation (and hence the amount of energy supplied). The efficiency is affected by the design of the wort kettle, particularly the heating surface area.

• Wort pH continues to fall during wort boiling. The drop in pH is mainly due to the reaction of calcium compounds with phosphates and polypeptides. These form insoluble compounds releasing H+ (hydrogen ions) so reducing the pH**.**

The kettle design will have a major influence. It has been found that more late-hop character persists in poorly agitated wort.

Wort boiling is a major usage of energy in the brewery and can account for 39% of the brewery's total energy. Steps are taken to make this process as energy efficient as possible by using heat recovery systems

Wort Boiling System:

Kettles are designed to provide a vigorous or 'rolling' boil and to be energy efficient. The main task is to provide sufficient energy input to ensure adequate turbulence and evaporation. A number of different designs have been made to achieve this. Two types of wort boiling systems are present –

• Internal Boiler

• External Boiler

Internal Boiler – In some kettles the heaters were upright and located in the centre to give improve recirculation currents. Some kettles also included base steam coils for preheating the incoming wort, and to minimise dead spots. This was developed further by the introduction of a "chimney" and "spreader plate" giving greatly improved recirculation and a greater surface area for evaporation.

The disadvantage of kettles with internal heating tubes is that the heaters tend to be difficult to clean with conventional CIP due to blind spots and are prone to corrosion. This can result in steam leaks into the boiling wort which are difficult to detect the repair. Wort circulation relies on thermal currents within the kettle and the turbulence over the heating surfaces is limited.

This results in high levels of fouling, and will require more frequent cleaning to ensure effective heat transfer in maintained.

The wort kettle which has the capacity of 140 HL consists of an internal boiler.

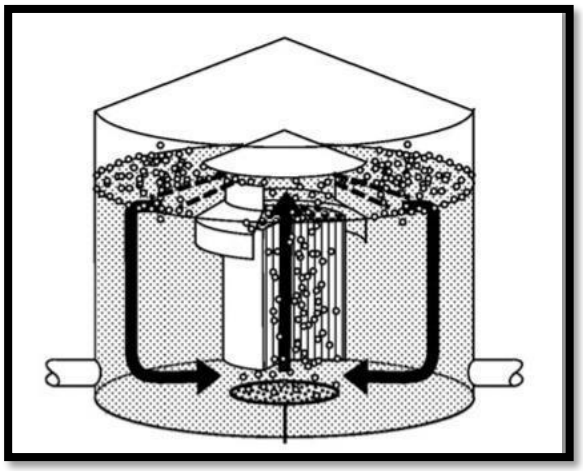


Figure17: Internal Boiler System

External Boiler:

Some of the latest designs use an external heater (external wort boiler). The wort is taken out of the kettle and passed through a shell and tube or plate heat exchanger for heating. The heat exchanger may be primed during the pre-boil stage, using a small circulation pump. In some installations when boiling starts, the circulation pump is by-passed. The wort then circulates using the natural “thermosyphon” effect. Circulation starts because hot liquid rises, cools down and therefore sinks again, etc. Incoming wort to the boiler is at 100°C and the outlet wort and superheated vapour from the boiler is around 105°C.

So the hotter liquid rises out of the boiler to the kettle. This saves pumping energy and limits the potential for damage to the "flocs" which would otherwise have to pass through a recirculating pump. The better heat transfer and turbulent conditions improve self-cleaning of the tubes. This allows between 8 and 16 brews (more in most recent designs) to be processed before a clean.

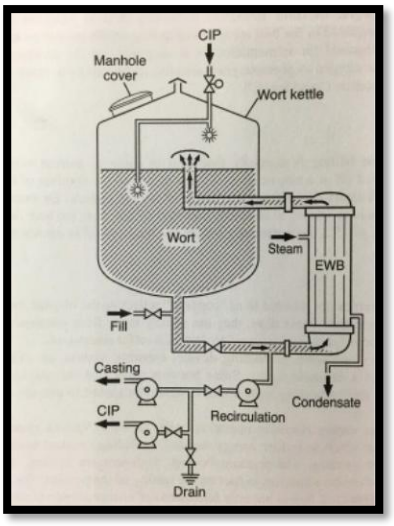


Figure18: External Boiler System

Boiling stabilizes wort by the sterilization and denaturation of enzymes production of bittering compounds, development of colour and protective compounds, flavour balance of hops and malt components.

Hops are dried female flower of hop plant Humulus lupulus. Approximately one quarter pound of hop flower is added per barrel of beer. Before boiling gravity must be tested range ~13. Sugar is added in last 10 mins of 1 hr boiling. Also, Brewtan B is added to sperate trub. After that final gravity is checked range~15.5. Then transfer to whirlpool Tank.

Chemicals Used in Wort Boiling:

Calcium Sulphate – This chemical is used to maintain the pH of solution.

Caramel– This chemical is used to reduce bitterness and encourage fermentation.

Redi Hops –Bitterness hops are generally added at the start of the boil, though where beer is to be packaged in clear (flint) glass, stabilized extracts only may be used to give bitterness, and no hops will be added at all during the boil. When brewing with whole hops or pelletized whole hops (i.e., not hop extracts), the principal hop volatiles lost during wort boiling are the hops oils. If these are present at too high a concentration, they may contribute a bitter, vegetable grassy flavour to the beer.

Brewtan B- Brewtan B is a type of brewing ingredient that used during wort boiling.

- **Whirlpool:** The whirlpool is probably the most widely used process for hot break separation. Whirlpools are used with hop pellets and extracts and not whole hop cones. It is a cylindrical vessel with a flat bottom into which wort, while still hot, is pumped through an inlet, laterally and tangentially. The inlet pipe is tapered so that the velocity of the wort increases. This configuration puts the wort in the whirlpool into rotation. The rotational movement causes the so-called "teacup effect", i.e., the same effect that can be observed when one stirs a cup of tea that still contains residues of tea leaves.

The tea residues collect in the middle of the cup. The same phenomenon is observed in the whirlpool. After some time, a break cone forms in the middle of the whirlpool. After the whole wort has been pumped into the whirlpool, a whirlpool rest takes place, usually taking 10 to 15 minutes, depending on the whirlpool design. The whirlpool rest should not be prolonged too much as the stability of the break cone might suffer. Disintegration of the break cone might be promoted. When the rotational movement has died down, the clarified wort can be removed from the whirlpool and sent to wort cooling. Whirlpools are used with hop pellets and extracts, but not with whole hops.

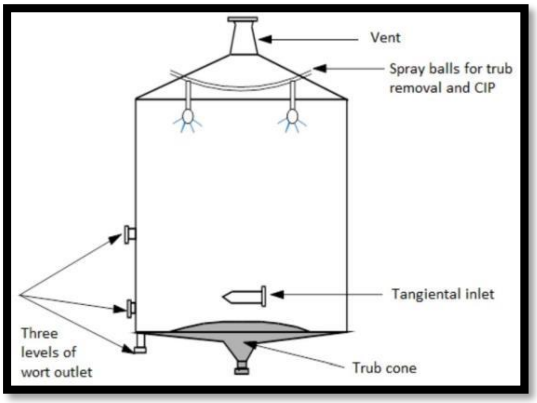


Figure19: Whirlpool design feature

Figure20: Whirlpool Machine

Wort Cooling:

After the whirlpool, the wort is brought down to fermentation temperatures before yeast is added. This is achieved by passing the wort through a plate heat exchanger. A plate heat exchanger has many ridged plates, which form two separate paths. The wort is pumped into the heat exchanger, and goes through every other gap between the plates. The cooling medium, usually water, goes through the other gaps. The ridges in the plates ensure turbulent flow. The heat exchanger can drop 90°C wort to 10 °C. After cooling, the wort is aerated to maintained the dissolved oxygen (DO) level from 8-10 ppm with the help of online DO meter. This level is maintained for proper yeast activity.

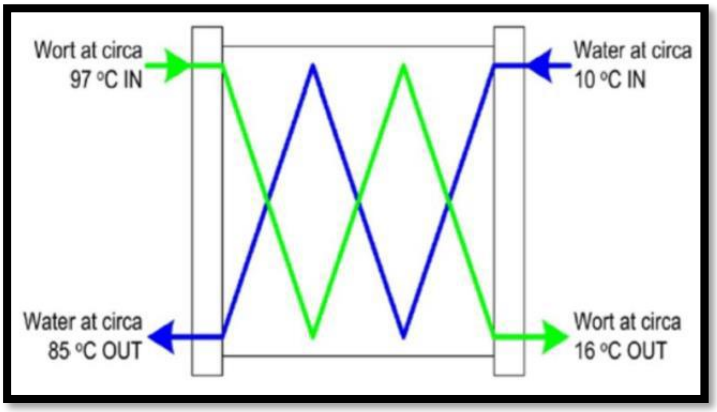


Figure21: Schematic diagram of Heat Exchange

Effects Of Cooling on Wort Constituents:

On cooling, wort proteins interact with polyphenols to precipitate as cold break. This material consists of very fine particles that are slow to settle and consequently are likely to survive into maturing beer. In combination, boiling and wort cooling remove 17-35% of the total protein content, depending upon the malt variety and hop product/variety used. Cold break formation is temperature dependent, only forming in significant quantities below 20-30°C and increasing dramatically in quantity as the temperature is further decreased. The removal of cold break can be enhanced by use of kettle finings.

Wort Cooling Methods:

The traditional form of wort cooler was the cool ship followed by a trickling cooler. Cold water passed through horizontal pipes and wort was fed over the pipes from the top. The wort was cooled by the cold water, and, in addition, more water evaporated from the wort film after the cool ship, again contributing to cooling. An open collection tank was placed at the bottom end of the series of pipes, allowing the wort to be runoff to FV and pitching. The large surface area allowed considerable wort oxygenation, but this system is prone to contamination pickup.

Nowadays the plate heat exchanger is almost exclusively used for wort cooling. This is because:

• Plate heat exchangers are very efficient and can cool the wort down in a short time. There is a large plate surface area for wort/coolant and the thin films of water / wort, and high levels of turbulence mean the heat transfer is very rapid.

• Nearly all the excess heat from the wort can be recovered to generate hot water for brewing and other production uses.

❖ WORT OXYGETION/AERATION

Optimum aeration of the wort is critical for uniform fermentation process. Aeration can be done either through the injection of sterile air or through the use of oxygen. And the addition point can be on either the hot or the cold side of the wort heat exchanger. It is important to provide oxygen in the wort so that yeast can replicate in the early stages of fermentation and thus ensure an adequate fermentation. If there is not enough DO in the wort, then there will be insufficient yeast replication, and this will lead to an inadequate fermentation.

It is normal to add the oxygen to the cold wort, since it rapidly reacts with hot wort to produce darker colours and encourage oxidation. The level of oxygen required depends on the yeast strain and is influenced by the wort gravity – high gravity wort requires a higher oxygen level. The amount of DO require is usually in the range of 7 – 18 mg/l oxygen.

Purpose Of Wort Oxygenation

The absence of sufficient oxygen in the wort for the yeast to make these sterols and fatty acids can lead to number of problems including: -

• **Sluggish fermentations** – high final pH and other flavour changes.

• **Early finish to fermentations** resulting in high final gravities.

• **Poor yeast growth** – insufficient for repitching.

• **Unhealthy yeast** (normally seen as low viability) which is not suitable for repitching.

• **Possible ability of contaminating bacteria** to grow faster than the yeast and spoil the beer.

Different yeasts and/or differing wort gravity require different amounts of oxygen. Wort oxygen levels of between 8ppm (air saturated wort) and 30 ppm (oxygen saturated wort) may be required depending on the wort gravity and yeast type used.

Wort Oxygenation Methods:

The wort cooling stage has proven to be the best time for controlling dissolving oxygen. This can be achieved through the use of air or from pure oxygen to a repeatable and measurable level.

It is usual to inject air or oxygen into the wort stream in-line. The amount of dissolved oxygen in the wort affects yeast growth so much that some form of control is required to guarantee consistency. The control can be achieved in a number of ways as discussed later.

The accuracy and repeatability of the control can be measured by using a dissolved oxygen meter. However, particularly in older breweries, wort may be aerated only once the wort is in the FV (fermentation vessels), where accuracy of addition (and measurement) is much less well controlled. Aeration can take place before or after cooling on wort transfer, or whilst the pitched wort is in the fermentation tank.

Aeration Versus Oxygenation:

Use of aeration or oxygenation is generally dictated by the level of dissolved oxygen required in the unpitched wort. It is not possible to dissolve more than about 10 ppm oxygen in cold wort when using air as the gas supply in a simple aeration system. However, it is possible to dissolve up to approximately 30 ppm oxygen in cold wort if using pure oxygen. Both these values are affected slightly by the cold wort temperature and wort gravity. The higher the wort specific gravity, the less oxygen will be dissolved. The higher the cold wort temperature, the less oxygen will be dissolved.

Typically, sales gravity beers need no more than air saturation i.e. about 9 ppm dissolved oxygen. High gravity beers may need oxygenation, up to 30 ppm. Very high gravity beers (e.g. SG of 80° +) may require additional oxygenation / aeration during fermentation to allow the wort to be fermented successfully.

Different yeasts need different levels of oxygen for adequate yeast growth. Since yeast growth has a direct effect on the level of higher alcohols and esters produced during fermentation, Some brewers use additional oxygenation / rousing during early fermentation to suppress the production of excessive levels of esters. In practice, levels of dissolved oxygen are finely tuned by each brewery for each quality to give fermentations that ferment on profile, give an acceptable flavour match and minimise excessive yeast growth and losses.

Air Injection – Advantages & Disadvantages:

Table 3

ADVANTAGE	DISADVANTAGE
Compressed air is inexpensive.	Air must be sterilised.
It will saturate to approximately the level required by many yeasts, although dissolved oxygen should still be measured to ensure consistent fermentations.	The large volume of N_2 introduced with the air is very difficult to fully dissolve and will pass through the fermenter, causing thick top foams. Aromatic flavour compounds can be sparged from the wort by these bubbles.

Oxygen injection – Advantages & Disadvantages:

Table 4

ADVANTAGE	DISADVANTAGE
Cylinder oxygen is free from microbes.	Extremely high levels of dissolved oxygen are possible unless a feedback control system from a dissolved oxygen analyser is used.
Only the quantity of oxygen required for the fermentation needs to be injected, to reduce costs.	
No large "nitrogen foams" will be created in the fermenter.	
Concentration levels are adjusted easily and accurately.	
Since oxygen is very soluble, usage costs are very low.	

Operating Principles Of Wort Oxygenation Systems:

The following discussion only relates to in-line wort oxygenation / aeration.

Factors that promote gas absorption include: -

• Low temperature.

• High pressure (e.g., by use of a back pressure control valve).

• Small gas bubbles at point of injection, to give very high surface area by using, for example, a venture system or a sintered stainless-steel candle.

• High levels of turbulence, which can be achieved by high flow rates, in-line mixers or plate heat exchangers.

YEAST ACTIVATION & MANAGEMENT

Brewing Yeast: Yeasts are eukaryotic (cells containing complex structures enclosed within membranes) microorganisms (organisms too small for the individual cell to be seen with the naked eye) which form part of the kingdom Fungi. Yeast is a single celled fungus. It is capable of growing anaerobically (i.e. in the absence of air) and breaks down sugars to release energy, producing carbon dioxide, alcohol and water.

In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water. Under anaerobic conditions, yeast grows much more slowly and to lower densities and glucose is incompletely metabolized to ethanol and carbon dioxide. It is important to realize that optimal yeast growth is distinct from fermentation. Therefore, the conditions and methodologies used for propagating and maintaining yeast need not be identical to those used for fermenting wort. The purpose of a yeast starter is not to produce an enjoyable fermented beverage but rather to produce a sufficient quantity of yeast for subsequent fermentation.

Top & Bottom Fermenting Yeasts: Brewing yeasts may be classed as "top-cropping" (or "top-fermenting") and "bottom-cropping" (or "bottom-fermenting"). Top-cropping yeasts are so called because they form a foam at the top of the wort during fermentation. An example of a top-cropping yeast is *Saccharomyces cerevisiae,* sometimes called an "ale yeast". Bottom-cropping yeasts are typically used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment well at low temperatures. An example of bottom-cropping yeast is *Saccharomyces pastorianus.*

Nutritional Requirements of Yeast:

When oxygen is available (aerobic conditions) to the yeast sugars are mainly converted into carbon dioxide and energy is created to produce new cells. Some unpleasant by-products are also produced.

When there is no oxygen available (anaerobic conditions), the yeast primarily utilises the sugar to produce alcohol(ethanol), carbon dioxide and a range of aromatic substances (esters and higher alcohols). While under anaerobic conditions new cells are not produced in large numbers.

Yeast has nutritional requirements other than just sugar including: -

• Protein or nitrogenous compounds in the form of amino acids. These are derived from the barley protein during malting and mash conversion.
• Lipids or fatty material. This is also supplied by the malt.
• Vitamins from the malt.

• Trace metals. Calcium is usually present in the brewing water. Zinc may be present in hop products, if not it can be added to the wort. Copper may also be present in hop products, but if stainless brewing plant is used, may have to be added to the wort.
• Oxygen is usually dosed into the wort. Oxygen is essential for healthy yeast growth and a large yeast population is required to ensure that the fermentation is healthy and fast.

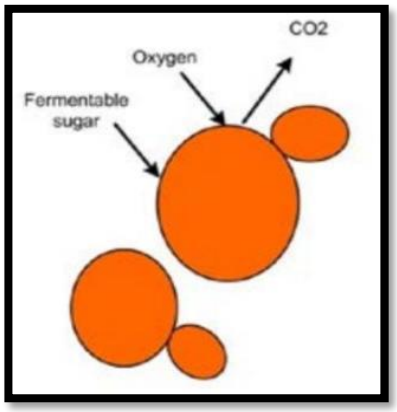


Figure 22: Aerobic Conditions – Increased Growth Rate

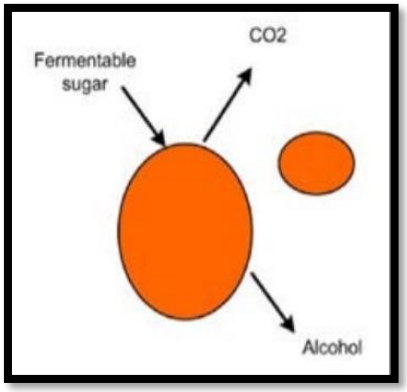


Figure22.1: Anaerobic Conditions – Increased Alcohol Production

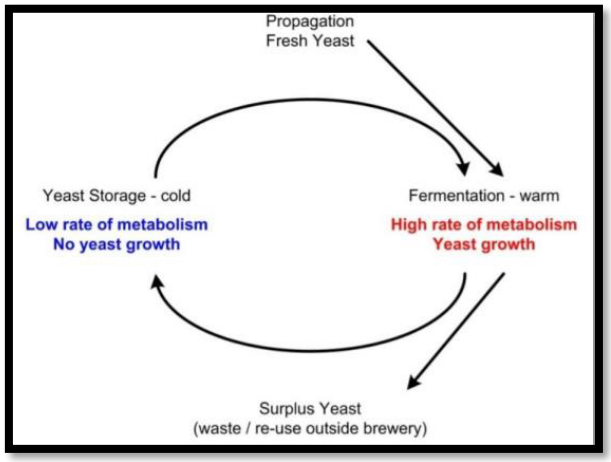


Figure 23: Brewing Yeast

Yeast must be stored carefully under hygienic cold conditions to keep it healthy and reduce the risk of growth of microbiological contamination.

Requirements Of Yeast Handling:

Yeast is the only living organism which should be found in most beers (there are a few notable exceptions which are not covered by these notes). At the start of the fermentation, it has to be added and mixed with wort which is at a suitable temperature for the strain of yeast & style of beer, is sterile, and has been oxygenated or aerated sufficiently to allow the yeast to grow. At the end of fermentation sufficient yeast must be separated using hygienic processes from the fermented wort (green beer), allowing the yeast to be re-pitched if necessary. The excess yeast (i.e., yeast not required for re-pitching) may be disposed of in a number of ways.

Periodically, normally defined by the number of times the yeast has been re-pitched, the yeast is to be replaced with a fresh batch of yeast grown from a single yeast cell (a new culture).

Main Processes Involved in Yeast Handling

• Introduction of replacement yeast strains – yeast propagation.
• Addition to wort – yeast pitching.
• Separation from fermented wort (green beer) – yeast cropping, including centrifugation and beer recovery from yeast slurries.
• Storage of pitching yeast between brews.
• If required, yeast sanitisation (acid washing).
• Disposal of surplus yeast – waste yeast handling. Excess yeast is typically sold on for yeast extract, health tablets, animal feed or the biochemicals industry, though of course it may simply be sent to liquid effluent systems or landfill.

Pure Yeast Culturing Procedures:

In most modern breweries, to maintain the yeast purity and the consistency of fermentations, yeast is typically propagated from a starter culture every 8 to 12 generations. Propagation starts in the lab and continues in special vessels with yeast count increasing between 5 and 10 times at each stage. Finally, the yeast is pitched directly into the fermentation vessel.

The principle of culturing yeast is to grow up a population of yeast from a single yeast cell. Because of the way that yeast multiplies (cells forming buds which break off and develop into new cells) each cell is a 'clone' of its parent. Consequently, a population grown from a single cell consists of identical cells known as a 'pure culture'. Yeast only grows well when it is in the presence of the sister cells, and so culturing is carried out in stages where the volume is increased at each stage.

Yeast Propagation Plant:

Yeast culture plants typically have the following features:

• Hygienic design and build, close to pharmaceutical standards.

• Provision for sterilising the medium, usually wortstandards boiling, though this is increasingly not required providing the quality of the CIP and sterilisation sequences can be shown to be adequate and hot wort is transferred to the vessels.

• Provision for very accurate cooling control.

• Provision for intense aeration or oxygenation.

• Provision for efficient but gentle agitation to ensure the yeast does not settle to the propagation tank bottom. Some systems rely on the oxygenation / aeration system to achieve the rousing rather than having a separate stirrer or recirculation system.

• A number of tanks for the different stages, each one being a larger volume than the previous one.

The diagram illustrates a yeast propagation tank with steam/cooling jackets. In this design, the air or oxygen is injected into the culture through a long lance extending through the spray head

to the bottom of the tank. This allows effective cleaning, (no shadows) and steam sterilisation of the air injection lance and the vessel. Other designs of oxygenation / aeration system use recirculation loops or simple sinters in the base of the vessel.

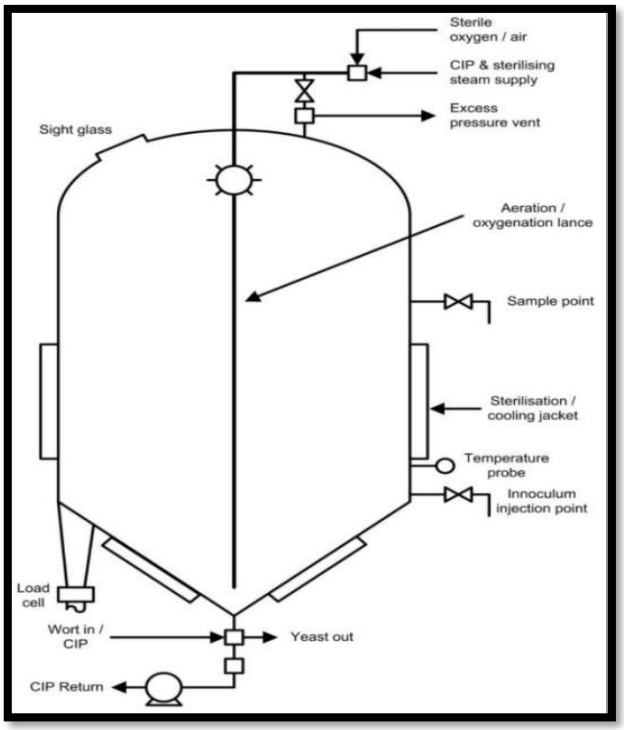


Figure 24: Yeast Propagation Tank

Yeast Cropping – Reasons & Times:

During the growth phase of fermentation, the yeast population grows approximately five-fold. This additional yeast can be cropped, and the best portions used for pitching into subsequent brews. Yeasts of different strains have different growth rates, and this factor needs to be considered when selecting suitable pitching yeast.

With top fermentations, the time of cropping can be used to select the best yeast for re-pitching. For example, the first and last skimming can be discarded, and the middle crop re-used. Yeast selection like this has enabled some ale brewers to maintain the same yeast strain for many years without culturing.

With bottom fermentations, yeast selection is more difficult, although selection of the most suitable yeast is possible by careful cropping processes. For example, the first purge from the cone can be discarded because it contains trub and a large proportion of dead yeast, and the final purge discarded because it contains some dead cell sand particularly low flocculence yeast, which may be considered unfit for blending back into the main beer stream – with associated loss of revenue and an increase in effluent & disposal costs.

The timing of yeast cropping is therefore to a large extent dependent upon the resistance of the yeast to autolyse when in a settled mass. Cone cooling in itself is of little use as the yeast is a very poor conductor of heat and will not allow the centre of the yeast mass to be cooled.

Yeast Removal Processes

Yeast for re-pitching must be cropped only from fermentations which meet specification for attenuation profile and diacetyl reduction. Wherever possible, microbiological status of fermentations should be available in time to prevent reuse of contaminated or cross-contaminated yeast.

At the end of fermentation in a cylindroconical vessel, the FV is typically cooled to around 4°C and the yeast settles out. The first portion of cropped yeast contains break and dead yeast cells and should go to waste / beer recovery. The next portion can be recovered if required for subsequent re-pitching. Yeast can be stored as a slurry in beer or pressed to recover the beer, and then either stored "dry", as a cake, or re-slurried with water. Some breweries store the yeast in the cones of the FV and pitch "cone to cone". Yeast can also be recovered from beer on transfer from FV to MV using a centrifuge, though normally the yeast recovered is not used for re-pitching but is sent to waste.

FERMENTATION :

The Production of Alcohol & Carbon Dioxide: Most living organisms respire aerobically. They use oxygen to convert sugars to carbon dioxide and water. This releases the energy. Some micro-organisms, including yeast, are able to metabolise anaerobically. They do not need to use oxygen to breakdown carbohydrate. However, the process, known as fermentation is inefficient and instead of carbon dioxide, water and energy, it converts those sugars into alcohol and carbon dioxide plus considerably less energy. The process starts when yeast is pitched into the wort and finishes when most of the sugar has been converted into alcohol and carbon dioxide.

The overall reaction for fermentation is:

$C_6H_{12}O_6$ -------► 2 (C_2H_5OH) + 2 (CO_2) + Energy

Glucose Ethanol Carbon Dioxide

(sugar) (alcohol)

Fermentation Phases:

1.Lag phase – Nothing happens to the wort specific gravity until the yeast has been pitched in. Yeast growth/reproduction and fermentation do not start immediately. The yeast cells metabolism becomes active following a period of relative inactivity during storage. The length of this phase depends on the type of yeast, the pitching rate, its health, in many cases including the age (number of times it has previously been repatched), and the conditions within the wort. The lag phase is caused by the yeast re-adjusting to the new environment and beginning to absorb and combine the nutrients and oxygen to give them everything required for growth and reproduction. The phase ends with the first cell division.

2.Growth (logarithmic or exponential) phase – The lag phase is followed by a rapid growth phase, starting between 6 and 12 hours after pitching. During the first part of this phase, the rate of cell division continuously increases. The specific gravity drops slowly at first whilst the yeast is growing and dividing. How long the accelerating growth lasts largely depends on the temperature. After about 24 hours the growth rate is constant and at a maximum. The cell numbers can double every 90 to 120 minutes.

Growth is only limited by physical parameters such as temperature and the availability of nutrients such as amino acids and by the amount of oxygen added to the wort. The specific gravity drops rapidly during the fermentation phase. The fermentable sugars are quickly converted into alcohol, large volumes of carbon dioxide are produced, and heat is generated. The pH of the beer also drops during fermentation.
Because specific gravity drops so rapidly and alcohol content increases so rapidly, this phase of the fermentation is often referred to as the Logarithmic (Log) or Exponential phase.

3.Retardation phase- The growth phase is followed by a retardation (late fermentation) phase which is limited by one or more of the following: -
• Available fermentable sugars. • Free nitrogen (amino acids).
• The increase in alcohol level.
• Settling or flocculation of the yeast. And to a certain degree, by lack of recirculation currents keeping the yeast in suspension.
4.Stationary phase – There is a balance between the number of newly formed cells and the cells which die. In the latter stages, sometimes called the declining phase, the rate of cell death exceeds the rate of new cell formation. The final stages of fermentation are slow, and it is where the yeast tends to "mop up" available nutrients that are available. Other biochemical reactions continue, the most important being the removal of diacetyl. Top fermenting yeast may be cropped at this stage. The beer may be reduced in temperature.

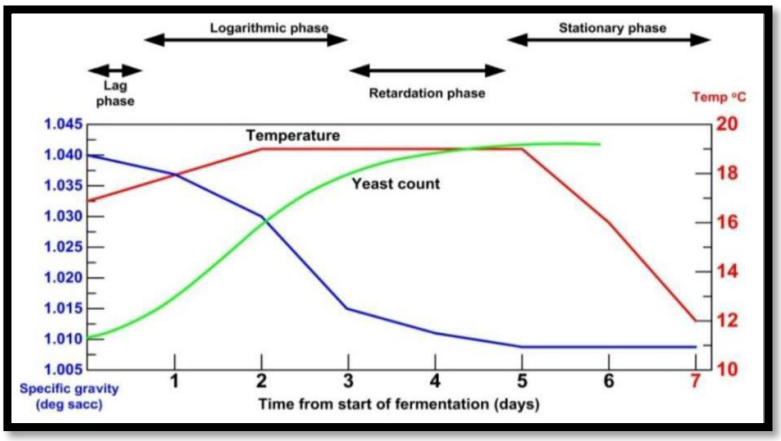


Figure25: Graphical representation of fermentation profile.

Factors Affecting the Phases of Fermentations:

• The yeast strain – including flocculation characteristics.
• The age of the yeast – older yeasts tend to produce more sluggish / abnormal fermentations.
• The wort oxygenation level.
• The wort temperature – including the control of the temperature rise.
• The wort composition in terms of nutrients –minerals, vitamins, amino acids.

Factors Affecting The Phases Of Fermentations:

• The pitching rate of yeast.
• The yeast strain – including flocculation characteristics.
• The age of the yeast – older yeasts tend to produce more sluggish / abnormal fermentations.
• The wort oxygenation level.
• The wort temperature – including the control of the temperature rise.
• The wort composition in terms of nutrients –minerals, vitamins, amino acids.
• The sugar concentration.

Metabolic Phase During Propagation & Fermentation

Carbohydrate Metabolism for Cell Growth and Energy Generation:

The dominating metabolic pathway of brewer's yeast during beer production is the formation of ethanol by consumption of wort carbohydrates. In general, alcoholic fermentation is an energy generation under anaerobic conditions, whereby glucose is metabolized to ethanol and CO2. The balance of energy is 2ATP/mol glucose.

Acetaldehyde serves as the hydrogen acceptor. In detail, glucose is transferred via glycolysis to pyruvate. The enzyme pyruvate decarboxylase secretes CO2 and acetaldehyde remains. Then alcohol dehydrogenase transforms the aldehyde to ethanol. Under strictly aerobic conditions the same pathways are taken up to pyruvate, but pyruvate is then changed to acetyl-CoA by the pyruvate dehydrogenase. In the following tricarboxylic acid cycle, the acetyl-CoA is used for energy generation. The hydrogen acceptor here is oxygen and 38ATP/mol glucose can be gained.

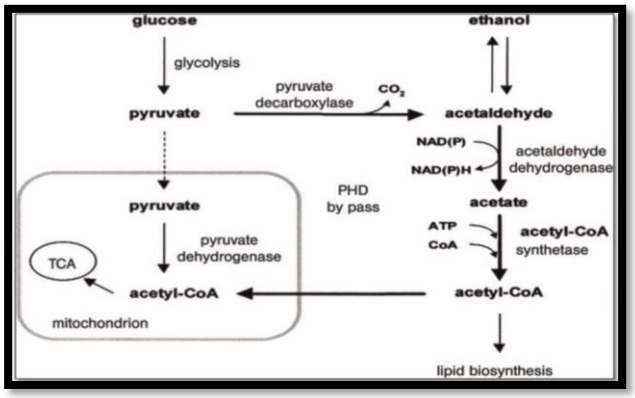


Figure 25.1: Carbohydrate metabolism

A very important regulatory phenomenon interacts in this pathway. The Crabtree effect refers to the occurrence of alcoholic fermentation (rather than respiration) of glucose under aerobic conditions because of higher sugar concentrations (Geiger et al., 2004). These sugar concentrations are always found under brewing conditions (e.g., working with wort). Thus, the Crabtree effect occurs constantly during propagation and the start of fermentation and is totally independent of aeration. Despite the low level of aerobic metabolism, yeast should also grow under brewing conditions. Acetyl-CoA as a precursor for biomass production has to be generated in a different way. Yeast metabolism takes a devious route. The acetyl-CoA is built via the so-called pyruvate dehydrogenase bypass. Here, pyruvate is transferred to acetaldehyde.

This is metabolized to acetate and then to acetyl–CoA via two enzymes: (i) acetaldehyde dehydrogenase and (ii) acetyl- CoA synthetase.

Phenolic Compounds:

A special flavour attribute of some German wheat beers is reminiscent of the aroma of cloves. The responsible substances behind this aroma are 4-vinylguaiacol and 4- vinyl phenol. For this type of beer, the flavour of 4-vinylguaiacol and 4-vinylphenol is out most important. The synthesis is regulated by the POF (phenolic off-flavour) gene. This gene can also be found in lager yeasts and even in wild yeasts, but it is only expressed in ale and wild yeast. A contamination with wild yeast results in a phenolic off-flavour in lager beers. For wheat beers, this flavour attribute is desired to a certain degree. It is synthesized by yeast out of the precursor's ferulic acid and coumaric acid. These acids are extracted out of the malt during mashing.

Formation of Sulphur Dioxide:

Sulphur dioxide is generated during primary fermentation by yeast. It is very important as natural antioxidant and enhances flavour stability of the finished beer. The component seems to be formed from intermediates in the pathway that reduces sulphate taken out of the wort. The sulphate enters the yeast by active transport and is reduced enzymatically to sulphite. After another reduction, the resulting sulphide is used for the synthesis of sulphur containing amino acids. When yeast growth ceases, no further sulphur containing amino acids are used and the sulphite is excreted out of the cell. Four phases of sulphur dioxide formation during primary fermentation can be distinguished. Phase 1: No sulphur dioxide excretion because sulphite metabolism is repressed due to a high level of sulphurs containing amino acids. Phase 2: Sulfurdioxide is used for the synthesis of amino acids. Phase 3: Yeast growth ceases, the synthesized sulphur dioxide is excreted. Phase 4: The total metabolism and the sulphur dioxide production come to a standstill. The sulphur dioxide synthesis is directly linked to yeast growth more yeast growth means less SO2, however, reduction of yeast growth to enhance flavour stability has to be assessed carefully.

Fermentation Vessels And Their Control:

The Basic Requirements of Fermenting Vessels
A fermentation vessel has a large number of functions, including those listed below:
• Its prime function is to contain the fermenting wort.
• It must protect the fermenting wort against ingress of foreign micro-organisms which could contaminate the fermentation.
• It must allow fermentation of wort at a controlled temperature by having provision for cooling.
• Sometimes it is considered essential to ferment at a constant pressure to control production of esters and to a lesser extent foam.
• It must allow CO2 produced during fermentation to escape or be collected sufficiently pure for re-use.
• It must allow, and preferably actively encourage mixing of the vessel contents to avoid layering and to disperse the yeast.
• It must be insulated (either directly or indirectly in a constant temperature atmosphere) to minimise influence of the ambient temperature affecting the wort temperature.

• The control must allow for elevation of the temperature of the fermenting wort to a desired value for warm maturation to allow effective use of the heat generated during the latter stages of the fermentation (free rise, mainly for diacetyl control).
• It must uniformly chill the contents to 1-3ºC for maturation and/or yeast sedimentation.
The design and control must allow / encourage sedimentation of the yeast and easy removal from the vessel while it is full.
• It is normally required to allow / encourage the solution of CO2 produced during fermentation in the beer, but not to excess.
• It must be easy to clean, preferably using CIP techniques.

The reasons for temperature control under brewery conditions yeast metabolises sugar by fermentation:

$C_6H_{12}O_6 \rightarrow 2(C_2H_5OH) + 2(CO_2) + Energy$

Glucose ethanol carbon dioxide

When an energy rich substance, such as glucose, is broken down to form a compound with a lower energy content (for example, ethanol), the excess energy (originally derived from photosynthesis when the barley grew) is released. Depending on whether the glucose is respired or fermented the amount of energy released is different. Approximately 25 times more energy is released during the respiration of glucose than during the fermentation of glucose by yeast. During fermentation under production brewing conditions, the amount of heat is approximately 586.6 KJ/kg of fermentable sugar. Wort is usually 70% fermentable. One hl of wort may contain 12 kg (12°P or 1048) of extract. Of this, 8.4 kg is fermented during the primary fermentation. Consequently 586.6 x 8.4 = 4927 kJ are produced by every hl of wort. This heat generated must be removed during fermentation by cooling in order to: -
• Ensure that the fermentation temperature is maintained at the set level which prevents the formation of unwanted flavours.
• Ensure yeast viability.
• Reduce fobbing.

Fermentation and filtration process:

Uni Tank

After full cooling at 4 °c

Pre Buffer Tank

Figure: Polyclar and stabiquick is added

Kieselguhr Doser

KMS and Shineaid is added

Hypro and Celite added

Bright Beer Tank

Ethanol and CO2 produced(350hl)

Post Buffer

Filtered beer

Filtrox

244 candle filter. De-aerated water is used for bed.

Yeast Culture

Boiled the wort to 100 °c and reduced to 15 °c then only the inoculum is added.

Yeast Storage

Yeast pitching at 4 °c

Yeast Propagator (1 hl)

Yeast Propagator (35

Yeast Propagator (7 hl)

Filtration

Beer that is intended to be clear (bright) is often assumed to be of better quality and there is much historical evidence to prove that this is often true. However, unfiltered, intentionally cloudy beer, including wheat beer, is still produced and enjoyed in many countries.

Matured beer will still have particles in suspension, mainly yeast but also smaller particles, mainly protein, unless it has been fined. There are three types of filtrations: -The purpose of 'rough' filtration is to remove all the particles that would make the beer cloudy.

The purpose of 'polishing' filtration is to remove all yeast and bacteria so that the beer is sterile. The purpose of 'stabilising' filtration is to prevent non-biological haze formation in package, which is the haze formed by the protein/tannin particles.

Traditionally, beer has been filtered using filter aids, predominantly Kieselguhr (KG). More recently however, new filter aids that are capable of being regenerated have been developed. These reduce powder purchase costs, waste disposal and improve safety aspects, albeit at the additional cost of a regeneration process

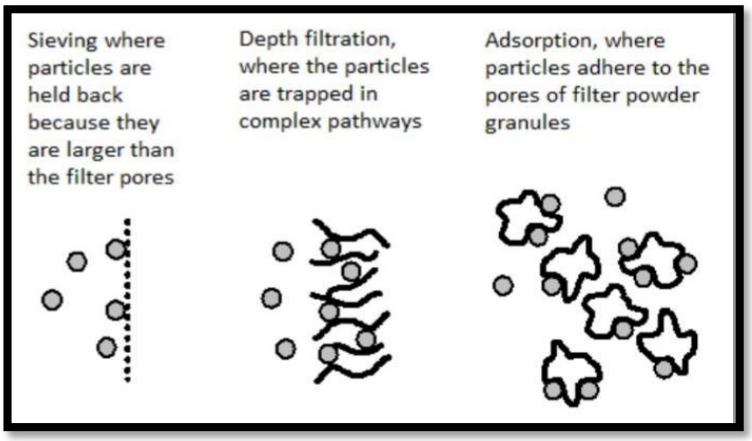

Sieving where particles are held back because they are larger than the filter pores
Depth filtration, where the particles are trapped in complex pathways
Adsorption, where particles adhere to the pores of filter powder granules

Figure26: Kieselguhr & regenerative powder filtration

Filtration using one or more filter "powders" uses the principles of absorption and depth, the technique as follows: -

A supporting medium (filter cloth or mesh) is pre-coated with filter aid. The precoat forms a depth filter, but is mainly required to bridge the gaps in the support medium (wire mesh, wedge wire, or cellulose fibre sheet) and prevent the support medium being blinded or allowing filter aid, yeast or proteins to pass through into the final filtered product. After pre-coating is complete, beer is introduced into the filter. To prevent the filter bed blinding with yeast and protein, additional powder, known as body feed is dosed into the beer as it runs into the filter. The yeast and protein particles are adsorbed by the body feed filter powder, gradually building up a layer of powder, yeast and protein until the filter is filled. The filter does not 'blind' because the body feed continually creates a new filter bed.

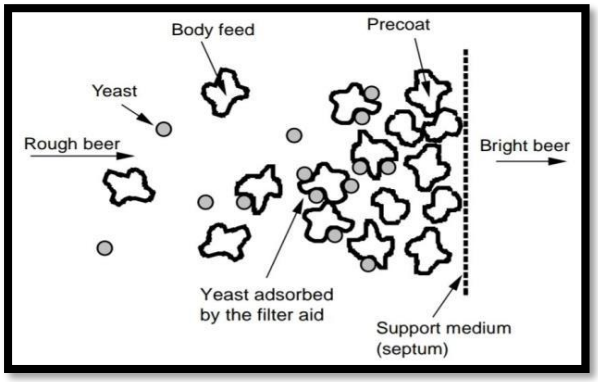


Figure 27: Filtration of Rough Beer

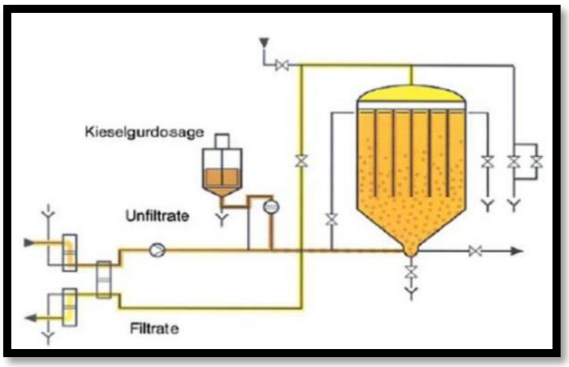


Figure 28: Filtration Process

Diatomaceous earth (DE) or kieselguhr is made from the skeletons of minute sea plants which have been kilned and milled to various grades of fineness. This is the most porous, rigid and effective filter aid. Kieselguhr can used for both precoat and body feed, depending on the grade (i.e. KG particle size). Kieselguhr is sometimes referred to by trademarked brand names such as Celite.

BEER MATURATION &COLD STORAGE

Introduction: The biochemical, chemical and physical mechanisms involved in flavour changes occurring during maturation are intricate and complex. Maturation includes all transformations between the end of primary fermentation and the final filtration of the beer. Classically fermentation and maturation are considered separate steps in brewing but in practice there is significant overlap. The main difference between traditional maturation systems in ale and lager brewing is that ales are conditioned by warm storage, holding the beer at 12 - 18°C, whilst lagers are conditioned at much lower temperatures (3 - 6°C). There are, of course, always exceptions to this traditional view.

Under warm maturation conditions, residual sugars are rapidly metabolized, and removal of "green" flavours is completed in 1-2 weeks (normally far less) depending on the type of beer, yeast strain, wort composition and primary fermentation conditions.

Maturation of lagers, particularly at low temperature, is significantly dependent upon the length of the lagering (ageing) period, the amount of yeast in suspension and the quantity of fermentable sugar in the young beer or added in the form of actively fermenting wort .Brewery conditioned and filtered beers, i.e. most keg, can and bottle beers, are matured in tanks where

flavours improve, the yeast is sedimented out (so aiding filtration), the beer is stabilised to ensure that it stays bright, and oxygen is purged / mopped up by the yeast to very low levels.
dependent, the rate being much higher at higher temperatures and higher yeast concentrations – hence the increase in temperature at the end of fermentation during some lager fermentations. Because the rate is dependent on the temperature and viable yeast cell concentration, maturation takes place before chilling of the beer and sedimentation of the yeast.

Cold storage & stabilization:

The purpose of cold storage improvements in beer flavour are achieved in two stages, warm maturation and cold storage. Yeast must be present for the improvements to occur. Cold storage takes place at 0°C or below and beer flavour continues to improve as the unpleasant compounds are reduced, though the rate of reduction is very much slower than at warm maturation temperatures. The raw materials from which beer is made, especially malt, contain protein material and tannins, which can combine together to form particles large enough to create hazes. This haze is termed non-biological haze because it is not caused by microbiological activity.
Non-biological haze can also be formed after filtration and packaging especially as the beer ages and in the presence of oxygen. A 'chill haze' can be formed if inadequately stabilized beer is stored in a cold place like a refrigerator because the remaining haze particles are less soluble at low temperature.
Other functions of cold storage include: -
• Oxygen reduction – by allowing residual yeast to mop up oxygen.
• Carbonation - by action of the residual yeast on any fermentable sugars, producing CO2.
• Stock holding – providing a buffer for periods of high demand.
• Blending – to improve consistency of final flavour etc. Ideally to be a routine operation, rather than as a need to bring out of specification beer into specification.
• Process aid reaction time – to allow sufficient time for reactions and settlement to take place.

Typical Times & Temperatures -

Ales typically -1°C for 1 to 4 days, though may be up to a week, particularly for small pack beers. Shorter residence times are most easily obtainable when using beer stabilisers such as silica gels or PVPP. Lagers -1°C for 4 days up to a few weeks, particularly dependent upon the origins of the beer. Traditional style beers are more likely to use longer times than modern recipes. Again, shorter residence times are most easily obtainable when using beer stabilisers such as silica gels or PVPP (where permitted).

General Principles Of Stabilization -

On transfer from FV (or MV) to cold maturation/storage vessel, the beer contains high levels of suspended solids, mainly of yeast and proteins. However, it also contains large amounts of protein and polyphenol precursors. These need to be largely removed before filtration to allow efficient filtration and the required shelf life. The period of cold maturation allows much of the

suspended yeast and proteins to settle out. The settlement / removal may be aided by use of centrifuges or processing aids.
At 0 to -2°C there is little yeast activity (although lager yeast can grow very slowly at this temperature). The principal changes are physical. Beer haze is formed during cold storage due to the combination of proteins and polyphenols, which either settle out in the storage tank or are removed during filtration. This occurs principally during cold storage and takes around 12 to 36 hours to form. However, the bonds are very fragile and almost instantly break if the beer is warmed up.
Particles, for example yeast and insoluble protein particles, will sediment out as long as they are heavier than the beer. The rate of clarification depends on the size of the particles, how dense the particles are and how far they have to fall. The simplest but lengthiest way of removing the yeast and haze particles remaining in the beer is to allow suspended matter to settle. Stoke's Law tells us the essential features of settling, namely that the rate of sedimentation is increased by: -

- Increasing the diameter of particles.
- Increasing the density of particles.
- Increasing the gravitational force.
- Decreasing the liquid viscosity

BRIGHTBEERTANK (BBT)

Tanks must be clean and sterile. They should have smooth internal surfaces. Tanks should be counter pressured with inert CO2 or nitrogen. Beer flow to the tanks should be at the correct flow rate without sharp bends which can promote fobbing. Inlet rate should be smooth to avoid gas breakout in the bottom of the tank. Correct high CO2 and DO levels by purging with oxygen free nitrogen through a sinter at the base of the vessel. Degassing to correct dissolved gas levels will cause some fobbing in the tank. Fob will remain on the vessel walls during emptying and could dry and cause a haze in the next filling if vessel rinsing is ineffective. Sometimes dried bits of collapsed fob fall back into the parent beer and cause similar rejects.
Tanks should be lagged or cooled using jackets. Usually, insulation is sufficient but temperature will rise so a maximum residence period of 2 -3days will be stipulated. Beer warming up will tend to lose dissolved CO2and N2, becomes more prone to micro infection and packaging warm beer leads to fobbing which may allow oxygen pick up and difficulties attaining the required fill levels. It is essential to recheck haze and dissolved gases, CO2, N2 and O2 every shift if the tank is still waiting to be packaged.

Objectives Of Bright Beer Tank:

- Carbonate the beer
- Childproof & stabilize the beer
- Modify & improve beer flavour balance

• Clarify the beer
• Blend & standardize the product

Figure29: Bright Beer

SUMMARY

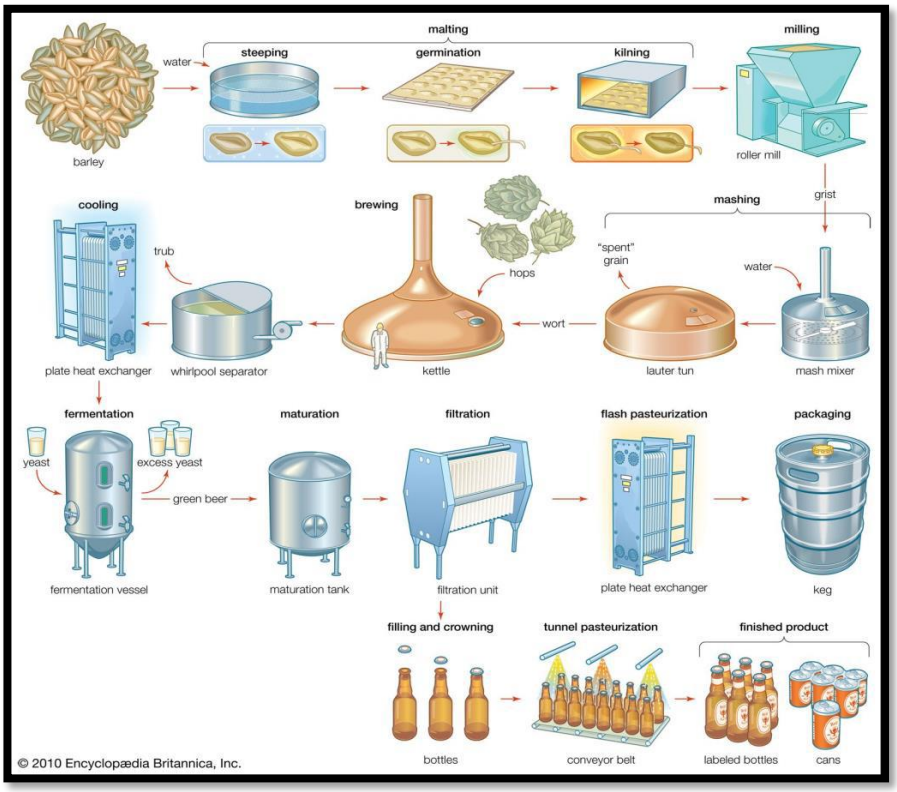
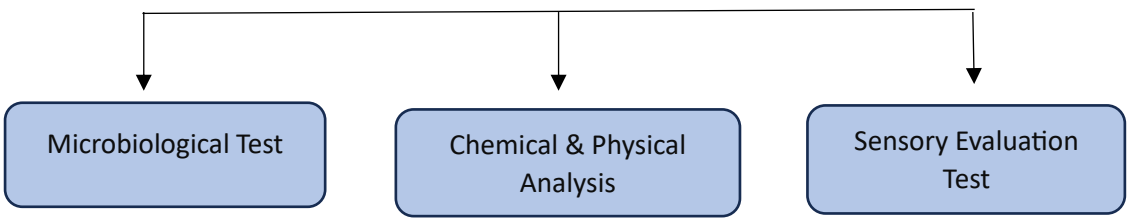


Figure30: Brief summary of total process

Quality Assurance



MICROBIOLOGICAL TEST

Microbial tests ranging from quantitative and qualitative significantly contribute to the identification of food and outbreak of foodborne diseases. These routine tests can be used to validate primary control methods, such as the Hazard Analysis and Critical Control Point (HACCP) system. Routine microbiological solutions cover the entire production process through to the QA/QC analysis of products while ensuring compliance with regulatory standards. They include:

- Hygiene monitoring for microbiological analysis of air, surfaces and personnel
- (HACCP management and ATP Testing)
- Water testing
- Pathogen testing
- Indicator organism testing
- Spoilage organism testing

BEER SPOILAGE MICROORGANISMS - RISKS AND CONSEQUENCES

Due to its low pH value, its high CO2 and alcohol content and the bittering agents it contains, beer generally does not provide good living conditions for most bacteria and is therefore usually not susceptible to contaminations. Nevertheless, some beer spoilage bacteria, like Lactobacillus spp., *Pediococcus spp*., *Pectinatus spp*. and *Megasphaera spp*., have adapted in a way that allows them to grow undisturbed even under these conditions. In most cases, these microorganisms do not pose a health hazard to humans, but can cause off-flavours and thus lead to the loss of entire batches and to significant damage to a company's image. If contamination is suspected or has already occurred, the entire process chain needs to be investigated as quickly as possible. The analysis process can be extremely time-consuming and can even lead to a production stop for a certain period of time. The economic losses in this case are enormous, which is why reliable and fast microbiological quality control is absolutely vital for the brewing industry.

Chemical And Physical Analysis:

- Determination of Colour in Beer

Purpose- Purpose of the Procedure is to follow appropriate Process for the Determination of

- Colour in Beer.
- Determination of Bitterness in Beer
- Analysis Of Alcohol%, Ae, Re, Og in Beer
- Determination of Haze in Beer
- Determination of PH Test:

It Is Measured by Taking the Sample Beer In A

Beaker And Then the Scale Is Dipped in It. The

Reading Is Shown by PH Meter Standard Value Of pH 3.5-4.

Figure 31 : different instrument used in laboratories

Beer Analysis as Per Bureau of Indian Standards (BIS) Ethyl alcohol (By Alcolyzer):

Ethanol is an organic chemical compound. It is a simple alcohol with the chemical formula C2H5OH. Ethanol is a volatile, flammable component and the active ingredient in alcoholic drinks. Ethanol is naturally produced by the fermentation of sugars by yeasts. Alternatively, by measuring the density of the starting material and the density of the products using a hydrometer the change in specific gravity during fermentation indicates the alcohol content. Modern digital density meters are based on oscillating U-tube principles. The tube usually a U-shape glass tube, is excited and starts to oscillate at a certain frequency depending on the filled-in sample. Through determination of the corresponding frequency the density of the sample can be calculated.

Presence of Methyl Alcohol:

Sample preparation-

1. $KMno_4$ solution- take 3ml of solid $KMNO_4$, add 15ml ortho phosphoric

acid, dissolve in it and finally make the volume 100ml with distilled

water, if require filter the solution.

2. Chromo tropic acid- Take 5-gram chromo trophic acid and dissolve it in

100ml of distilled water and filter the solution.

Test method-

· Take 50ml beer sample add 50ml of water.

· Distilled the solution and collect 2ml in test tube.

· Add 2-3ml of $KMNO_4$ solution in it.

· Keep it in deep fridge for 30 minutes.

· Add sodium bi sulphate pinch.

· Wait until sodium bi sulphate colour disappear.

· Add 5ml of chromo tropic acid.

· Add 10ml of conc.H_2SO_4 slowly using dropper.

· Put the test tube in hot water and allow it to boil.

· Remove the test tube and see the colour.

CO2 in Bottle:

This invention relates to a method of determining the gas content of gas-liquid solutions under pressure, and is more particularly adapted to determine the percentage of co2 in beer, the principal object of this invention is to provide a method of this character which will enable the gas.

Diacetyl Test:

- First 100ml of sample was taken in a beaker.
- Then 3.5ml di sodium hydrogen phosphate was added. Solution was mixed and distilled at 75℃.
- 1ml hydroxide ammonium chloride was added to 10ml.20ml distillate was taken sample and set at oil bath till volume 3-5ml.
- Other 10ml distillate was added to it and 1ml disodium hydrogen phosphate was added to it.
- It was set in the spectrophotometer at 220nm to find the absorbance value which gives the equivalent concentration of the diacetyl.

Bottling Hall Check:

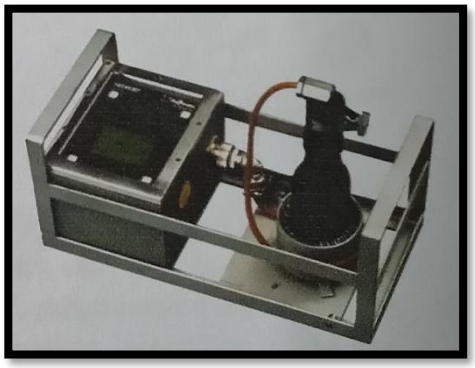
Net Volume of Beer:

- Take an empty bottle weight
- Take a weight of filled beer bottle

Weight of beer = (weight of beer bottle-weight of empty bottle)

Volume= (weight of beer bottle -weight of empty bottle)
Specific gravity (1.007)

PU or Pasteurization Unit:



PU value is the duration (in minutes) of the entire pasteurisation process, at a defined temperature. In pasteurization application, especially for drinks, one encounters the term pasteurization units -PU. One PU is defined as exposing a product at 60℃ for 1 min. Generally, the PU range should be in between 12-30, if less then under pasteurization and if more than over pasteurization.

SENSORY EVALUATION TESTS:

Sensory analysis can provide a qualitative measurement (is beer A sweeter than beer B) as well as a quantitative measurement (on a scale of 1 to 5, beer A is a 4 while beer B is 2 with regards to sweetness). This measurement could be made by trained personnel (a sensory panel) or by consumers, depending on the aim of the study. The aim of the study can further be divided into objective analytical quantity such as flavour intensity or a subjective value such as preference. This basic principle along with asking the right question to the correct sample group at the right time allows breweries to make key decisions about their products and the responses of their consumers to their product.

Flavour Attribute in Beer

Table 5

Following are the flavours of the beer generally found by the testers in the beer industry:

COMPONENT	DESCRIPTION
Control	No Flavour
Iso-Amylacetate	Banana, Pear Drops, Fruit, Stick of Rock
Ethyl acetate	Acetone, Glue, Nail Polish
Acetaldehyde	(green) Apple, Kiwi Fruits
Floral	Floral
Hops	Floral, Hops
Chlorophenol	Antiseptic, Disinfectant, Hospital
Smoky/phenolic	Pharmacist, Chemicals, smoked sausages

Iso-Valeric Acid	Vomit, Old Cheese, Sweat
Diacetyl	Butter, Caramel
H_2S	Rotten Egg, Vulcanic
Light-struck	Burnt, Coffee, Rubber
DMS	Natural Gas, Cooked Cabbage, sprouts
Catty/Cassis	Catty, Cassis, Black Current Leaves
Nonenal	Cardboard, Papery, Pencils
Oxidation	Grape Juice, Rye Bread
Anisole	Earth/soil, aquarium, Musty, Fungus (wine cork)
Sour	Sour
Sweet	Sweet, Sugar
Bitter	Bitter
Metallic	Blood, Iron, copper(coin)
Rough/Astringent	Alum, dry

Food Safety Measures in Plant:

OPRP-Operational Pre-Requisite Program:

We Have Three OPRP in our Plant. These Are

- Wort Aeration in Brew House.
- Carbonation of Beer in Tank Farm.
- Chlorine-Di-Closing in Water Treatment Plant.

CP-Critical Control Point: All CCPs are in bottling hall.

- EBI: No CCP-checking bottles.
- Filler: No CCP bottle burst at the time of filling.
- Pasteurization: No CCP bottle pasteurization.

(P.R.P) Pre requisite programme:

Prerequisite programmes: it provides the basic environmental and operating conditions essential for the safe production of food.

Examples of prerequisite programmes include:

- Pest Control
- Cleaning and Sanitation (CIP)
- Housekeeping, Personal Hygiene
- Standard Operating Procedure (SOP)
- The Designs and Construction of Food Premises
- Personal Hygiene
- Procurement and Logistics
- Raw and Packing Material Storage

- Finished Goods Storage.

Food Safety Hazards

A food safety hazard is an agent or condition that could potentially cause an adverse human health effect.
Agents are either in or on food and can be either physical, chemical, or biological.

REDUCTION OF OFF-FLAVOUR (DIACETYL) FROM BEER BY YEAST MANAGEMENT SYSTEM

Introduction:

In the theatre of brewing, yeast is viewed as a "supporting actor". But unlike the more commercial leading actors such as water, malt and hops, yeast appears not only once but in many successive productions. Further, with fermentation being the longest chunk of the process, yeast is on stage longer. Finally, and critically, as an agent of change, yeast facilitates the most dramatic event in the process of transformation of wort to beer. Yeast is a single celled microorganism that reproduces by budding. They are biologically classified as fungi and responsible for converting fermentable sugars into alcohol and other by products.

❖ **Yeast Life Cycle**

The life cycle of yeast is activated from dormancy when it is added (pitched) to the wort. Yeast growth follows four phases, which are somewhat arbitrary because all of the phases may overlap in time:

- Lag period
- Growth phase
- Fermentation phase
- Sedimentation phase

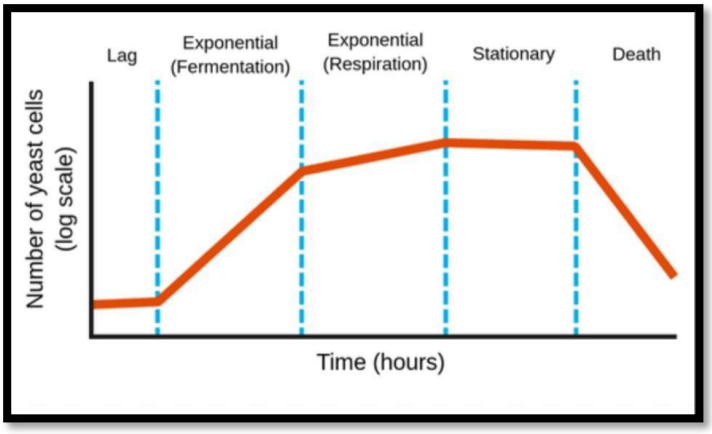


Figure: Growth Curve of Yeast at log scale (Number of yeast cells vs time)

❖ Flocculation

The flocculation characteristics of yeast are of great importance. The term "flocculation “refers to the tendency to form clumps of yeast called flocs. The flocs (yeast cells) descend to the bottom in the case of bottom-fermenting yeasts or rise with carbon dioxide bubbles to the surface in the case of top-fermenting yeasts. The flocculation characteristics need to be matched to the type of fermentation vessel used a strongly cropping strain will be ideal for skimming from an open fermenter but unsuitable for acyl Indro-conical fermenter. A phenomenon shown by brewing yeast is very important for the fermentation performance and following beer production steps flocculation. The ability of yeast to build cell clusters and settle to the bottom of the tank can be positive or negative for the process. If the yeast settles down too early, not enough yeast cells remain active to reduce diacetyl. If the flocculation is too weak and too many yeast cells stay in suspension, filtration and haze problems may occur. At present, the exact mechanism behind flocculation remains uncertain. Several theories have been proposed and some factors influencing the process have been determined (e.g. zinc and calcium). The cell wall seems to play a major role in flocculation; its composition is discussed below.

❖ Attenuation

Attenuation refers to the percentage of sugars converted to alcohol and carbon dioxide, as measured by specific gravity. Most yeasts ferment the sugars glucose, sucrose, maltose, and fructose. To achieve efficient conversion of sugars to ethanol (good attenuation) requires the yeast to be capable of completely utilizing the maltose and maltotriose. Brewing yeasts vary significantly in the rate and extent to which they use these sugars. Lager strains are often better at utilizing maltotriose than there are counterparts. The degree of attenuation obtainable exerts a great influence on the organoleptic properties of the resultant beer and, consequently, is one of the determinant factors in the process of yeast selection.

❖ Flavour Component

The selection of the yeast strain itself is perhaps one of the most important contributors to beer flavour. Different strains will vary markedly in the by-products they produce: esters, higher alcohols, fatty acids, hydrogen sulphide, and dimethyl sulphide. The yeast strain must also be capable of reproducible flavour production.

❖ Yeast Morphology and Chemical Composition

Brewing yeast is mostly round or ovoid. Partly elliptical or cylindrical cells can also be observed. They are of very regular shape and size. Cells measure 5–10μm in diameter, 3–1μmin width, and 4–14μm in length. The values are imprecise because the size of the cell depends greatly on the physiological state (e.g. prior to budding, yeast cells can reach 3 times the volume of regular cells). Like other living cells, yeast is composed mainly of water. The non- aqueous cell material is polymeric. Carbohydrates, proteins and nucleic acids made up of the six elements carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur form the bulk of the material with a large range of low molecular weight organic compounds and inorganic ions making up the remainder.

REVIEW OF LITERATURE

❖ DIACETYL

The compound responsible for buttery or butterscotch flavours that sometimes arise in beer can be controlled if you understand the mechanisms that contribute to its production. This review of the basic processes behind diacetyl formation and reduction will help to understand how to keep the diacetyl level in the beer at or below the acceptance threshold for the style. Diacetyl 2,3-pentanedione are important contributors to beer flavour and aroma. Organic chemists classify both as ketones, and diacetyl is usually called 2,3-butanedione in the literature. Sometimes these two ketones are grouped and reported as the vicinaldiketones (VDK) content of beer. Brewers' awareness and acceptance of both diacetyl and 2,3-pentanedione have changed dramatically over the past four to five decades. A1952 report, for example, stated that the average diacetyl level of American commercial beer was 0.33 mg/l, more than three times the flavour threshold of 0.10 mg/l.Today the average is near 0.05 mg/l. Some notable exceptions exist. Some stouts can have levels as high as 0.60 mg/l, and a few British pale ales have diacetyl levels near 0.30 mg/l. Diacetyl levels in beers brewed by microbreweries and brewpubs tend to vary considerably, ranging from 0.03 mg/l to more than 1.0 mg/l in cases I have investigated. The presence of diacetyl is usually indicated by a buttery or butterscotch tone. In fresh beer the flavour can be confused with that of caramel malts. Given time it is easy to distinguish the two diacetyl tends to be unstable in most beers and can take on raunchy notes. The flavouring imparted by caramel malts, on the other hand, tends to be stable. The flavour threshold of 2,3-pentanedioneis near 1.0 mg/l; it usually imparts flavours that recall honey. This compound can be found well above threshold levels in some Belgian ale, where it is considered a natural flavour constituent for this style. It occurs less often in other beer styles and is regarded as a defect.

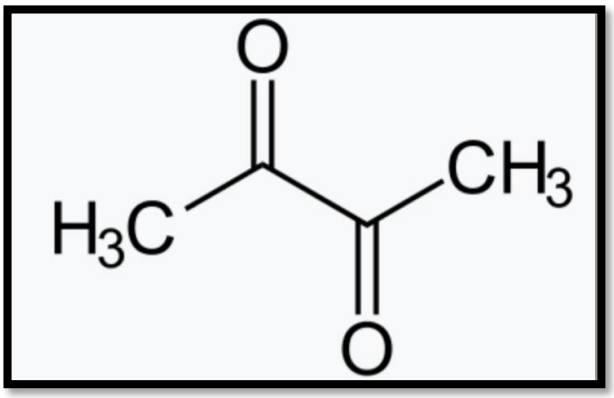


Figure32: Chemical formula of Diacetyl

❖ Diacetyl Formation

The study of diacetyl and beer began with Pasteur's fundamental work in the 1870s. Using microscopy, Pasteur found that what we know today as lactic acid bacteria were responsible for many off flavours in beer. The term sarcina sickness is used to describe this effect. Apparently, the involvement of diacetyl in sarcina sickness was discovered early, but it was not until 1939 that Shimwell linked this compound with the taste and smell of butter. Even today it is estimated that20% of beer drinkers do not detect the presence of diacetyl even at rather high concentrations.

During the early period, the only known mechanism of Diacetyl formation was bacterial infection caused by unsanitary conditions. Practical brewers believed that some other factor must be involved, because buttery tones occasionally showed up in beer brewed in impeccably clean environments. This work pointed to culture yeast as a major player in both the production and the reduction of diacetyl Inoue and his colleagues at the Kirin Research Laboratory in Japan also made a major contribution by identifying acetoacetic acid as the precursor to diacetyl. This work was followed by a large number of papers in which various facets of diacetyl formation and reduction were studied. Wainwright's excellent 1973 review article contains 149 references. Although a number of factors affect diacetyl formation in beer, the basic middle pathway shown in Figure 1 is the one used for each. The dominant carbon flows down the left most branches, leading to ethanol production. Most beers contain between 30,000 and 50,000 mg/l of ethanol, so a significant amount of pyruvate is processed in this manner. Because the Flavors threshold for diacetyl is 0.10 mg/l, as light diversion of the carbon flow to the middle pathway can profoundly affect the flavour of finished beers. Note that this pathway also competes with yeast assimilation and the utilization of the amino acid valine. The practical significance of this is discussed in the section "Wort and Proteins" below. A similar pathway is involved in the production of 2,3- pentanedione, except that different compounds are involved. The precursor is acetohydroxy butyrate, and the competing amino acid is leucine. A number of factors lead to diacetyl formation, but only one reliable method can reduce diacetyl levels, enzymatically reduction by yeast. Acetone, the intermediate product, has a rather unpleasant, musty taste, but because it has a flavour threshold of 3.0 mg/l its effect is not nearly as damaging to beer flavours as an equivalent amount of diacetyl. The final product, butanediol, is neutrals far as beer flavour is concerned

❖ Bacterial Influences

The bacteria that can directly promote diacetyl production consist of Gram-positive cocci (Pediococci) and select strains of Gram-positive rods (Lactobacillus). The effect of using these bacteria is easy to identify both bacteria also produce lactic acid, and the net effect is a rather raunchy butter tone with an unmistakable acid after taste. In both amateur and commercial brewing of yesteryear, infections from unsanitary equipment were not uncommon. Today, with the availability of highly effective sanitizers, infections tend to occur only in sloppy and poorly managed operations. It has been my experience that in modern operations, infections, when they occur, happen through pitching yeast. It is unnecessary for fully developed pitching yeast to be sterile. Practical experience has shown that as long as bacteria relevant to beer are kept below the level of 1-10 cel*ls per 10 million yeast cells, the finished beer will remain unaffected*.

❖ MATERIALS REQUIRED

✓ UV-Spectrophotometer
✓ Distillation Apparatus (Condenser, Round bottom flask, collector, bent tube, air tight glass wares)
✓ Pipettes
✓ Volumetric flasks
✓ 25ml graduated cylinders
✓ Oil bath
✓ Yeast slope
✓ Zink sulphate heptahydrate
✓ Fermenter
✓ Incubator, Autoclave
✓ Slides, Cover Slip Petri plates, Petri plates
✓ Microscope
✓ Bottle turner

Reagents

✓ Hydroxylamine hydrochloride (NH2.OH. HCl) 8% aqueous solution.
✓ Disodium hydrogen phosphate heptahydrates (Na2HPO4) 8.5% aqueous solution.
✓ Saline water (0.85% NaCl).
✓ CuSO4 Solution.
✓ Y.M.C.A (Yeast Malt Agar with Copper Sulphate) Media.
✓ W.L.N(Wallenstein Laboratory Nutrient) Media.

Stock Solution: Dimethyl glyoxime: 0.1349gm of pure dimethyl glyoxime was dissolved in distilled water and the volume was made up to 1000ml by adding distilled water, such that the resulting solution has the concentration equivalent to 100mg per litre of diacetyl.

Working Solution

From the stock solution, 20ml was taken and diluted with distilled water and the volume was made up to 100ml, such that the resulting solution has the concentration equivalent to 20mg/litre of diacetyl.

❖ METHODS

➢ STARTER CULTURE PREPARATION

▪ **Yeast Propagation** 1. The yeast cells from the slope were removed by using sterile saline water.
2. It was inoculated in two flasks containing 500ml sterile wort each.
3. It was incubated at 25-27°C for 48hrs with constant oxygenation.
4. Then the two 500ml cultures were inoculated in to two flasks containing 5 litres sterile wort and incubated at 20-22°C for 48hr with constant oxygenation.

5. The two 5 litres culture were inoculated to first yeast propagator containing 1HLof wort heated with 100°C and was cooled to 16-18°C and incubated at 16- 18°C for 24-36hrs.
6. The 1HL cultured was transferred to second yeast propagator containing 10HL wort heated with 100°C and was cooled to 13°C with constant oxygenation.

▪ Yeast Budding Study

A drop of yeast sample was taken over a glass slide. A piece of cover slip was kept over it and viewed fewer than 100X binocular Olympus microscope, for the morphological study.

▪ Fermentation

10HL cultured from yeast propagator was pitched to culture brew of 100HL at 10- 12°C. Online aeration was given during wort collection and the temperature was maintained 10-12ºC during fermentation. The fermentation was carried out 7-8 days at temperature 10-12°C till the end gravity and pH 5.5. The fermentation medium composed of amino acids, carbohydrates and proteins.

▪ Wild Yeast Study

Y.M.C.A Media (T.C.M. Media):

24gms of Y.M.C.A was taken in to 1000ml and was autoclaved at 121°C for15mins. 25ml of CuSO4 was added to the media before pouring to the plates. The media was poured to plates and allowed for solidification. After the solidification of media, the sample was poured over it and was kept in incubator for 24hrs.

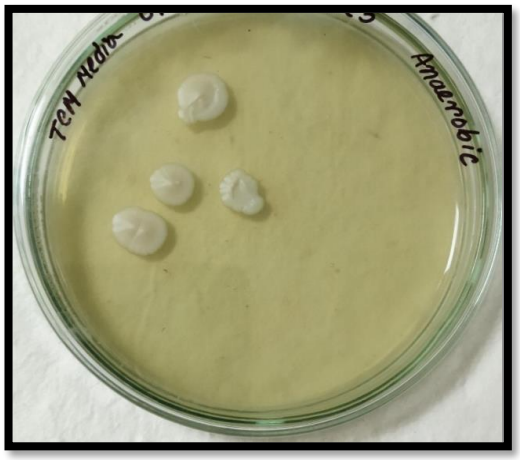


Figure 33: Wild Yeast on Y.M.C.A.

Diacetyl Estimation:

Standard Curve:

1. 0, 1, 2,3,4,5 ml of dimethyl glyoxime working solution was taken in 25 ml graduated cylinders.
2. 1ml of hydroxylamine hydrochloride and disodium hydrogen phosphate was added to each tube and the volume was made up to 10ml.
3. Absorbance was taken at 230 nm and the graph was plotted between absorbance vs blank to get standard curve and the regression factor was calculated.

Sample Measurement:

1. 100ml of undegassed beer was taken in 500ml round bottom flasks.
2. 3.5ml of 8.5% Na2HPO4 was added in to it.
3. Distillation was started with tip of the delivery tube dipping in to 2ml of distilled water.
4. The distillate was collected in 25ml graduated Pyrex cylinder to 18ml and the volume was made up to 20ml by distilled water.
5. 10ml of the solution was transferred to another 25ml graduated Pyrex cylinder and labelled one cylinder for sample and another for blank.
6. 1ml of 8% Na2.OH. HCl was added to the cylinder labelled “sample”.
7. Both the cylinders were heated in oil bath at 80°C for 15mins and were cooled in room temperature.
8. 1ml of 8% Na2.OH. HCl was added to the cylinder labelled “blank.”
9. 8.5% Na2HPO4 solution was added to both the cylinders. The volume was adjusted to 10ml by distilled water and was mixed thoroughly.
10. The absorbance was taken of cylinder labelled sample vs cylinder labelled blank at 230nm.
11. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.

▪ Study On Reduction of Microbial Contamination During CIP

CIP (Cleaning in Place) plays a significant role in each and every Brewing Industry. It is basically a cleaning process of UTs which comes to play after every batch of fermentation. To reduce microbial load, a sterilant known as Divoson Forte is generally used as final rinse in CIP process.

▪ Study on Antimicrobial Activity of Divoson Forte:

1. Five sample bottles were taken & labelled as Blank,0.1%,0.2% ,0.3%,0.4%.
2. 400ml Soft water which is used for CIP was collected in five numbers of 500mlsample bottle.
3. To increase the microbial load in the water samples some different kinds of microbes such as Acetic Acid Bacteria, Lactobacillus Bacteria, Wild yeast has been added manually.
4. All the bottles were mixed thoroughly by using the bottle turner for 10 minutes.
5. 0.5ml,1.0ml,1.5ml,2.0ml Divoson Forte were added to 0.1%,0.2% ,0.3%,0.4%labelled bottle respectively.

6. Again, all the bottles mixed properly for 10 minutes.
7. Using the Membrane Filter technique, all five samples were filtered through the0.4micron membrane filter using a filter funnel and vacuum system.
8. The filter paper was placed in respective WLN plates and incubated for24hours at 30 degrees Celsius.

❖ RESULTS

▪ Starter Culture & Yeast Propagation

Starter culture was selected, and yeast was cultivated in Yeast Cellar with all sterile and optimal conditions for multiplication.

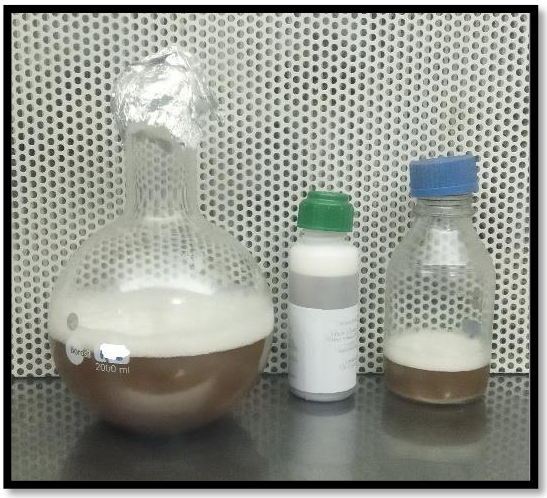


Figure 34: Yeast Stain

Figure 35: Yeast Cellar (CB Flask)

The yeast propagation was carried out at temperature 16-18°C in yeast propagator

Yeast Budding Study:

Morphological examinations were performed using the media wort as the nutrient for the yeast. The budding of yeast was observed under 100X magnification of Olympus binocular microscope on haemocytometer.

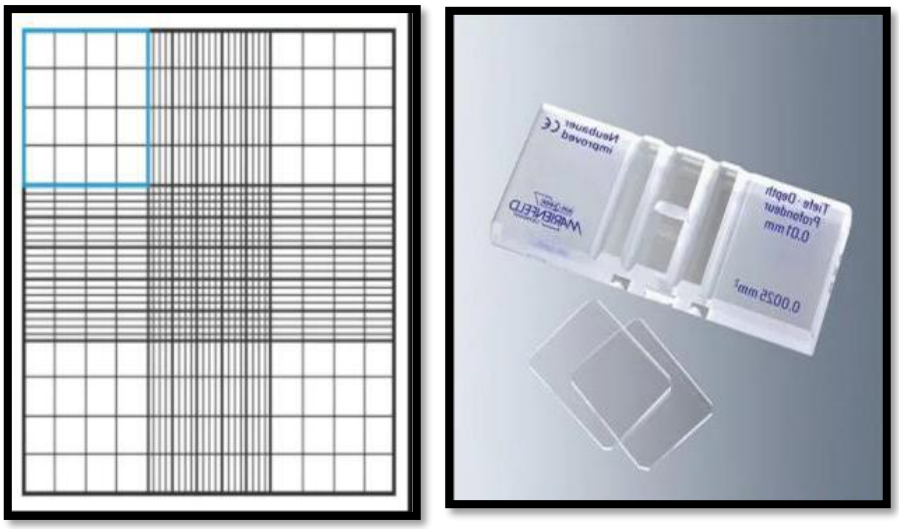


Figure 36: Haemocytometer

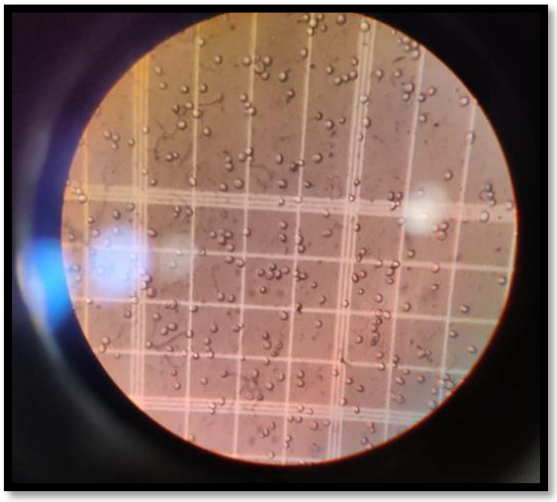


Figure37: Bud formation of Yeast (Microscopic View)

Fermentation:

Yeast from propagator was introduced to YPT (Yeast Pitching Tank). After 7-8 days of fermentation the substrate was converted into beer.

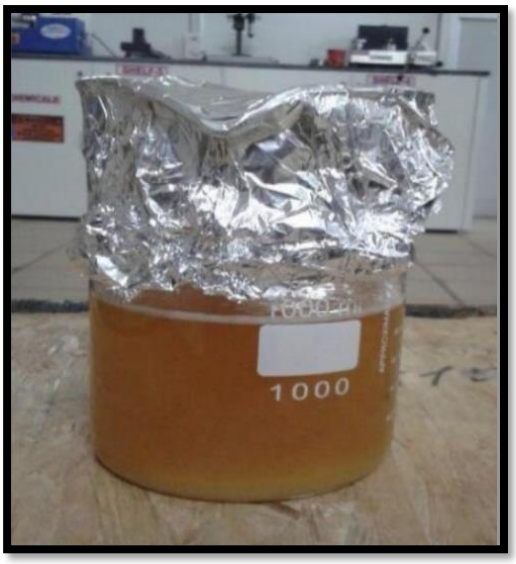


Figure 38: Sample collected from Fermentation Vessel

Wild Yeast Study

On completion of incubation, numerous colonies of Saccharomyces wild yeast observed on T.C.M. media.

Figure 39: Wild Yeast in TCM Media

After fermentation, yeast batches harvested and stored in YST. A single batch of yeast can be cropped and harvested up to 6thgeneration.

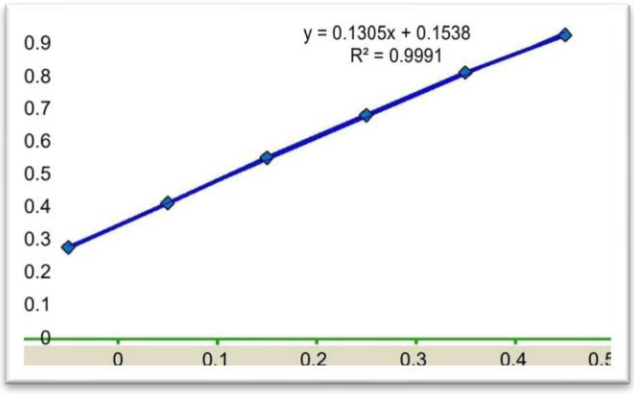
Figure39.1: Yeast Storage Tank

Diacetyl: Calculation For Regression Factor-

Table 6

Serial no	Volume of standard solution(ml)	Concentration(ppm)		Distilled water(ml)	Absorbance at 230 nm	Slope	Regression factor
1	0	0	1 ml of 8% Na2HPO 4	8	0.141	1.304	0.7663
2	1	0.1		7	0.278		
3	2	0.2		6	0.413		
4	3	0.3		5	0.552		
5	4	0.4		4	0.682		
6	5	0.5		3	0.812		

Absorbance at 230 nm



Diacetyl (in ppm)

Figure 39: Standard Graph for Diacetyl

CALCULATION:

The sample was measured under UV spectrophotometer at 230nm and it was observed 0.0894nm. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.

Diacetyl (ppm) = Absorbance × Regression Factor

= 0 0.0894 X 0.7663

= 0 0.0685 ppm

It was found that the diacetyl amount in beer was 0.0685 ppm.

Discussion

Diacetyl is naturally produced by all incentive during turmoil and smells like Butterscotch. Diacetyl is naturally produced by all incentive during turmoil and is also “reabsorbed” by incentive cells. Increased diacetyl or diacetyl that is not reabsorbed may be a result of high flocculating incentive, weak or shifted incentive, over or under oxygenating, low turmoil temperatures and weak or short boils. It's generally regarded as an excrescence when detected in lagers. Some spirits, and alkies likewise, desire small quantities in ales.

still, rescuer headache and vomiting will arise after drunk, If diacetyl is more in beer also sickness in the mouth and lingo. The ketone group increases toxin in beer. The entire beer aroma will suppress by VDK. If VDK is more in beer also it can not be consumable.

incentive that's largely flocculent may fall out of suspense before it gets a chance to absorb the diacetyl, using medium flocculation incentive should give the incentive a good chance to absorb diacetyl. Always use high quality incentive and avoid weak or conceivably shifted beaches that may be unable of handling diacetyl duly. Allow incentive to begin original growth with the use of a incentive starter. Sufficient oxygen is to be supplied for incentive growth, but avoid over oxygenating especially after pitching incentive. Allow enough time for incentive to completely raise at applicable temperatures.

Conclusion

Beer is alcoholic libation consumed each over the world. It reduces the threat of heart complaint, helps the order, packed with nutrition containing vitamin B, VitaminB12, Riboflavin and also rich in silicon which helps to strengthen the bones, reduces threat of diabetes, threat of cancer, and increases brain health if consumed in moderate. However, also similar health problems arise as rescuer headache, puking after drunk, if diacetyl is there in beer. The beer will smell like adulation. The entire beer aroma will suppress by diacetyl.

For Natural enzyme activation in barley, Malting process is done. For high recovery Exogenous enzymes are used that provides that helps maintaining the body of the beer, that improves quality of beer. Because the natural enzymes in malt is not capable to give good recovery and due to use of Adjuncts used in brewery, adjuncts have less enzyme activity. Proper boiling of wort can develop good colour and flavour to the beer

Yeast recovery is done after the fermentation process for recovery of yeast after each generation for reuse of yeast up to sixth generation, so that yeast pitching cost can be reduced. and it is use to six generation only because after sixth generation yeast is not that much viable that it can produce required amount of alcohol in beer processing. If we will use that yeast, it will affect quality of beer like change in flavour

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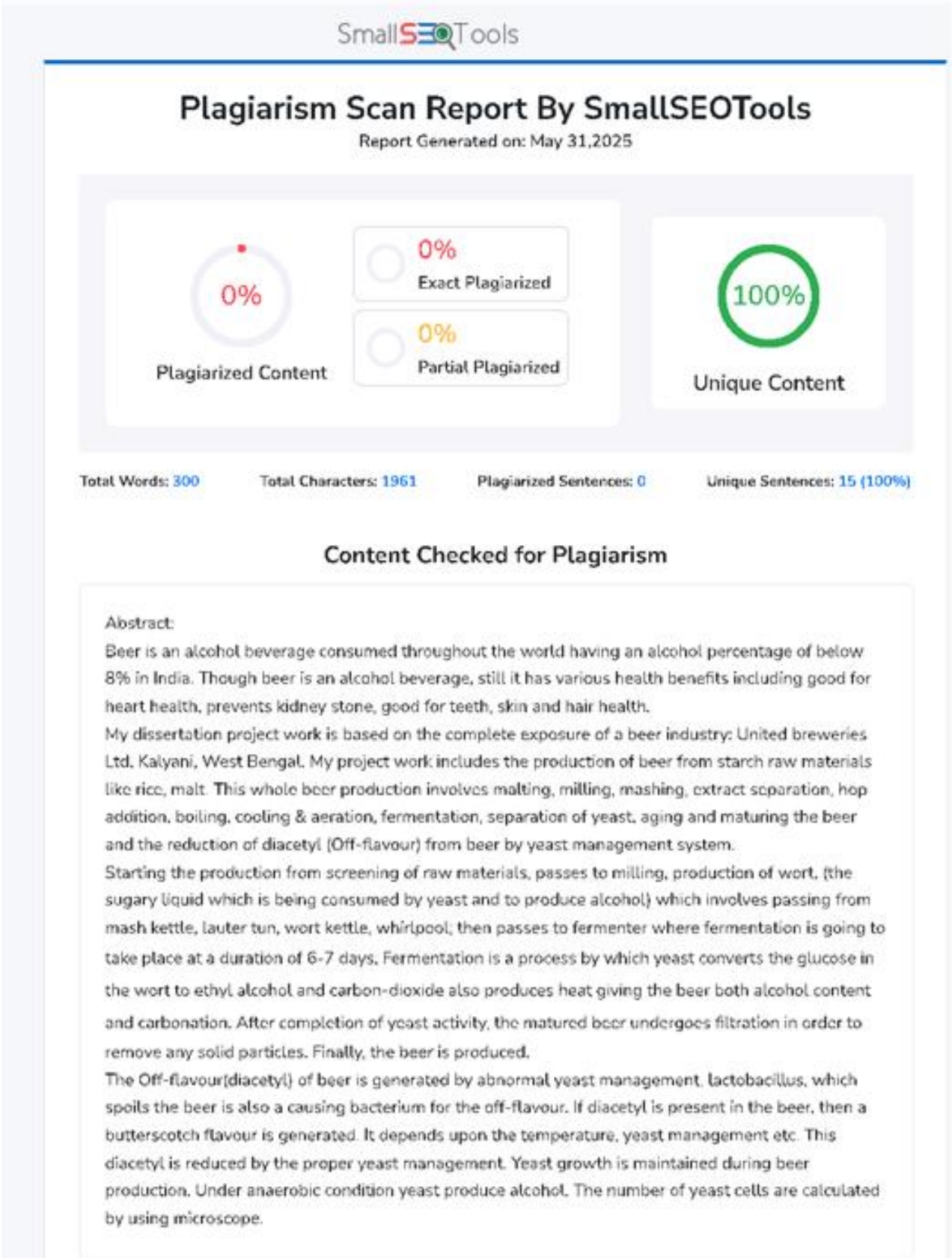

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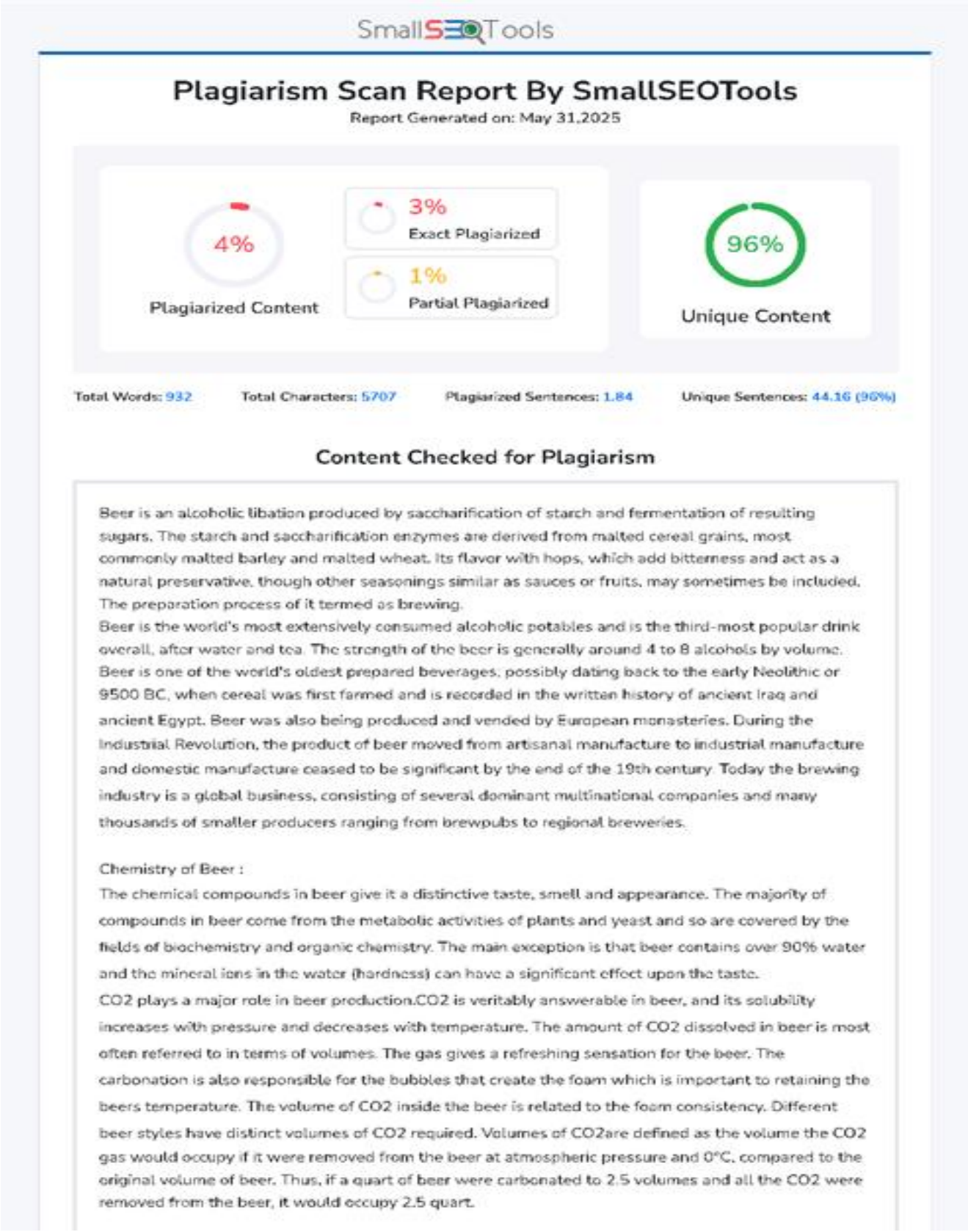
Abstract:
Beer is an alcohol beverage consumed throughout the world having an alcohol percentage of below 8% in India. Though beer is an alcohol beverage, still it has various health benefits including good for heart health, prevents kidney stone, good for teeth, skin and hair health.
My dissertation project work is based on the complete exposure of a beer industry: United breweries Ltd. Kalyani, West Bengal. My project work includes the production of beer from starch raw materials like rice, malt. This whole beer production involves malting, milling, mashing, extract separation, hop addition, boiling, cooling & aeration, fermentation, separation of yeast, aging and maturing the beer and the reduction of diacetyl (Off-flavour) from beer by yeast management system.
Starting the production from screening of raw materials, passes to milling, production of wort, (the sugary liquid which is being consumed by yeast and to produce alcohol) which involves passing from mash kettle, lauter tun, wort kettle, whirlpool; then passes to fermenter where fermentation is going to take place at a duration of 6-7 days. Fermentation is a process by which yeast converts the glucose in the wort to ethyl alcohol and carbon-dioxide also produces heat giving the beer both alcohol content and carbonation. After completion of yeast activity, the matured beer undergoes filtration in order to remove any solid particles. Finally, the beer is produced.
The Off-flavour(diacetyl) of beer is generated by abnormal yeast management, lactobacillus, which spoils the beer is also a causing bacterium for the off-flavour. If diacetyl is present in the beer, then a butterscotch flavour is generated. It depends upon the temperature, yeast management etc. This diacetyl is reduced by the proper yeast management. Yeast growth is maintained during beer production. Under anaerobic condition yeast produce alcohol. The number of yeast cells are calculated by using microscope.

INTRODUCTION



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Beer is an alcoholic libation produced by saccharification of starch and fermentation of resulting sugars. The starch and saccharification enzymes are derived from malted cereal grains, most commonly malted barley and malted wheat. Its flavor with hops, which add bitterness and act as a natural preservative, though other seasonings similar as sauces or fruits, may sometimes be included. The preparation process of it termed as brewing.
Beer is the world's most extensively consumed alcoholic potables and is the third-most popular drink overall, after water and tea. The strength of the beer is generally around 4 to 8 alcohols by volume. Beer is one of the world's oldest prepared beverages, possibly dating back to the early Neolithic or 9500 BC, when cereal was first farmed and is recorded in the written history of ancient Iraq and ancient Egypt. Beer was also being produced and vended by European monasteries. During the Industrial Revolution, the product of beer moved from artisanal manufacture to industrial manufacture and domestic manufacture ceased to be significant by the end of the 19th century. Today the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers ranging from brewpubs to regional breweries.

Chemistry of Beer :
The chemical compounds in beer give it a distinctive taste, smell and appearance. The majority of compounds in beer come from the metabolic activities of plants and yeast and so are covered by the fields of biochemistry and organic chemistry. The main exception is that beer contains over 90% water and the mineral ions in the water (hardness) can have a significant effect upon the taste.
CO2 plays a major role in beer production.CO2 is veritably answerable in beer, and its solubility increases with pressure and decreases with temperature. The amount of CO2 dissolved in beer is most often referred to in terms of volumes. The gas gives a refreshing sensation for the beer. The carbonation is also responsible for the bubbles that create the foam which is important to retaining the beers temperature. The volume of CO2 inside the beer is related to the foam consistency. Different beer styles have distinct volumes of CO2 required. Volumes of CO2are defined as the volume the CO2 gas would occupy if it were removed from the beer at atmospheric pressure and 0°C, compared to the original volume of beer. Thus, if a quart of beer were carbonated to 2.5 volumes and all the CO2 were removed from the beer, it would occupy 2.5 quart.

• Proteins (MW>5000)
• Iso- -acids
• Metal ions
• Gums (-glucans, xylenes)

Factors affecting foam stability (negative)
• Lipids (negative) • Amino acids • Alcohols

Raw Material:
Requirements for Production
1. Malt
2. Adjunct
3. Water
4. Yeast
5. Miscellaneous additives

MALT:
Malt is barley or other cereals like wheat which has been germinated under controlled conditions followed by draying to terminate the germination before an actual new plant has developed and this process known as malting. The house where this process is going on is termed as Malt house. The aim of the malting is to loosen up the endosperm by degrading the endosperm cell walls and produce enzymes for further degradation of the content of the endosperm cells during mashing.

Barley is the principal grain used in brewing for a number of reasons:
[] The plant can be grown in many parts of the world ranging in latitude from near the polar circles to the equator.
[] Its uniform and convenient size makes it easy to handle on an industrial scale.
[] The grain contains 60 – 65% by dry weight of starch. Together with proteins, enzymes, vitamins and minerals, the grain provides a complete package for yeast nutrition.
[] It contains sufficient lipids for yeast metabolism under anaerobic conditions, in some other grains excessive levels of lipids can have deleterious effects on beer processing or quality.
[] The husk is relatively tough and can form a filter bed in the brewhouse.

The malting process is 3 types:
Steeping: In this process the germination is initiated by increasing the moisture content of the grains from about 12% to about 45%. Barley is soaked in water to simulate the conditions that start germination or growth. This is carried out in a steep tank. During steeping the grain is aerated, (normally by draining and drawing air through the wet grain bed, before resoaking) to reduce the numbers of grains dying off due to "drowning" and to increase the rate of water uptake. The barley is usually steeped and aerated a number of times – at the end of steeping the moisture content of the barley should be around 45%, dependant on the type of malt being produced.
Germination: In this process the grains are allowed to germinate for 3-7 days depending on the vitality of the grains. Along with the growth of the roots, the endosperm is modified, and active enzymes are built up. On completion of steeping, the barley seed is allowed to grow. During germination two major changes occur:
Firstly, hormones stimulate the production of enzymes in the aleurone layer.
Secondly, these enzymes start to act. During malting they will break down the gummy cell walls and break down the protein matrix inside the starch containing cells. This breakdown releases the starch granules making them accessible for conversion into sugar. The changes taking place during germination are called 'modification'. The maltster can influence the degree of modification during malting by controlling the moisture content of the grain, its temperature and the time allowed for germination.

Plagiarized Sources

[] The plant can be grown in many parts of the world ranging in latitude from near the polar circles to the equator.
https://www.academia.edu/40574810/BAHIR_DAR_INSTITUTE_OF_TECHNOLOGY_FACULITY_OF_CHEMICAL_AND_FOOD_ENGINEERING_DEPARTMENT_OF_CHEMICAL_ENGINEEERING_INTERNSHIP_REPORT_BY_NAME_ID_No

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What does malt contain?

Like most commodities, malt is taken for granted. However, the myriads of components it provides us with is remarkable. Important chemicals contributed to the brewing process by pale malt include starch, protein, amino acids and peptides, phosphate, polyphenols, melanoidins, O- and N-heterocyclic compounds, lipids and sterols, beta-glucan, vitamins, metal ions, and enzymes. In addition, malt husk material provides the filter medium for mash tun and lauter tun separation systems.

Starch: The starch granules comminute from the malt make up the greatest portion of the extract. This cereal starch is in granular form with two forms: Amylopectin (70-80%) & Amylose (20-30%).

Malt Adjunct: Adjuncts are un malted grains (such as corn, rice, rye, oats, barley and wheat) used in brewing beer which supplement the main mash ingredient, often with the intention of cutting costs, but sometimes to create an additional feature, such as better foam retention, flavours or nutritional value or additives.

Water: Beer consists of more than 92% water. Sourced water is treated to brewing quality standards. The composition of the water that is to be used in brewing is very important. The quantity and character of dissolved salts are much significant while pH is secondary interest. Water containing calcium and magnesium sulphate with a permanent hardness of 200-300 ppm is usually stable. The characteristics of good water for brewing are a pH of 6.5-7.0 less than 100 ppm of calcium and magnesium carbonates, 250-500 ppm calcium sulphate, 200-300 ppm sodium chloride and less than 1 ppm iron.

Yeast: During fermentation, yeast cells convert cereal-derived sugars into ethanol and CO2. At the same time, hundreds of secondary metabolites that influence the aroma and taste of beer are produced. Variation in these metabolites across different yeast strains is what allows yeast to so uniquely influence beer flavour. (Saccharomyces cerevisiae) yeast is used in the production of beer in kingfisher.

MISCELLANEOUS ADDITIVES:

Gypsum – Used to increase the hardness of water, it reacts with phosphate from malt and reduces the pH toward the optimum for mashing. Calcium ion also stabilized many enzymes.

Calcium Chloride dihydrate– Used to increase the hardness of water.

CaSO4 dihydrate- pH control and yeast nutrition.

Neutrase: As a protease enzyme to break down proteins in wort

Termamyl SC: Gelatinization of adjuncts.

Lactic Acid: pH adjustment for enzymatic activity.

Attenuzyme For RDF control.

Brewtan B: For flavour stability, clarity, inhibit oxidation, trub separation.

alpha and beta acids are not soluble in water, in order to allow them to contribute to the bitterness of the beer, they must be modified. The bitterness, flavor and aroma of hops are released into the wort through boiling. The timing of additions of hops to the wort is called a hopping schedule. The amount of bitterness imparted to the wort in called utilization. There are four basic classifications of hop use. Bittering, flavoring, finishing and dry hopping.
Sugar: Sugar is also an additional carbon substance to the yeast, and it is also a malt adjunct. It doesn't require any enzymatic activity because yeast can use directly. Approximately 80-90% of the weight of these malt adjuncts is extracted into the wort.
Water Testing & Required Parameters
Table 1
Serial No Parameter Specification Unit
1 Raw water CLO2 0.8 Ppm
2 Ro water CLO2 >0.2 Ppm
3 Ro water hardness >=60 Ppm
4 Soft water CLO2 >0.2 Ppm
5 Soft water hardness <10 Ppm
6 RO 2 permeate 30 KL
7 RO – TDS permeate 40-50 Ppm
8 RO reject TDS 1100-1200 Ppm
9 RO – PH ~6.7
10 Borewell water withdrawal >1220 KL/day
11 RO feed pump flow rate 40 M3/h

Brewing Process
MALT MILLING PROCESS :
The malt and adjunct are important from other supplier by local transport and store in Silo. There are Four silos of capacity100 MT (rice) & 100(malt silo)200 MT (Malt Silo),200 MT (Malt Silo). There is a blower provision to circulate the air inside the silo, to avoid the dampening effect of the malt. It passes from silo through different cleaning process.

Roller malt milling:
In roller mill there are six rolls for milling process and gapes between rolls are respectively 1.4:0.9:0.65.
Brew House Operation
The brew house consists of the following unit:
- Adjunct kettle
- Mash kettle I, Mash kettle II
- Lauter Tun I, Lauter Tun II
- Holding Tank I, Holding tank II
- Wort kettle I, Wort kettle II
- Whirlpool

[] MASHING:
ADJUNCTKETTLE

Gelatinization of broken rice is done here. It's a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen& oxygen) to engage more water. This irreversibly dissolves the starch granules. Penetration of water increases randomness in the general starch granule structure and decreases the number and size of crystalline regions. Crystalline regions don't allow water entry. Heat causes such regions to become diffuse, so that the chains begin to separate into an amorphous form. During gelatinization, water act as plasticizer.

Plagiarized Sources

Yeast: During fermentation, yeast cells convert cereal-derived sugars into ethanol and CO2.
https://sharpbeer.com/article/is-beer-a-raw-fermentation-product

Variation in these metabolites across different yeast strains is what allows yeast to so uniquely influence beer flavour.
https://tonero.me.uk/beercomponents.htm

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Enzymes used in Adjunct Kettle :

• Termamyl SC 4X – This chemical contains -amylase enzyme which helps to break the alpha bonds inside the starch molecules.

• Calcium Sulphate –This chemical helps to maintain the pH of solution.

• Gypsum

MASH KETTLE

Ground malt and solid adjuncts (grist) are mixed with a set volume of water to achieve a specified temperature. The mash is then allowed to stand for a period of time, typically around an hour, during which the enzymes in the malt convert the starch to sugars to produce a sugary liquid called wort. Depending on the type of malt, it may be necessary to heat the mash to specified temperatures so different enzymes work close to their optimum temperature. During mashing:

[] Cell wall components may be broken down to release the starch (non-isothermal mashes only, where the endosperm has not been fully broken-down during malting)

[] Proteins are broken down to amino acid.

[] Starch is broken down and converted to sugars.

[] The pH drops

Mashing is the process by which sweet wort is prepared. It involves mashing in, the mixing of the milled grist and water at the correct temperature and in the correct proportions to obtain the mash. After a period, with or without temperature changes, during which the necessary biochemical changes occur, the liquid sweet wort, which contains the extract, is separated from the residual solids, the spent grains. The purpose of mashing is to extract the soluble sugars, dextrins and inorganic substances from the malt grains in addition to converting the insoluble material (starch and protein) to a fermentable form. It's probably the most important process in the brew house. Here saccharification test is tested.

α

Enzymes used in Mash Kettle:

o Calcium chloride –This is used to correct the mineral deficiencies in water is to be used for brewing.

o Calcium sulphate – This chemical acts as a buffering agent to maintain the ph.

o Attenuzyme pro – This chemical contains glucoamylase. This helps in saccharification, catalysing reverse reaction in which the dextrose molecules get combined to form maltose and isomaltose. This chemical also helps in achieving high real degree of fermentation (RDF).

Lautering

LAUTER TUN

It is a process in brewing beer in which the mash is separated into the clear liquid wort and the

residual grain. 5 mins rest during lautering of mash for bed formation, lautering usually consists of 3 steps: mash out, recirculation, and sparging. Mash out is the term for raising the temperature of the mash to 76 - 78°C. This stops the enzymatic conversion of starches to fermentable sugars, and makes the mash and wort more fluid. Recirculation consists of drawing off wort from the bottom of the mash, and adding it to the top The mash itself functions much as a sand filter to capture mash debris and proteins. Sparging is forcing water through the grain to extract sugars from the grain. Here water is spreading two halves', one is 1st sparging using of 70-75 Hl water by 90-100Hl wort collected then 2nd sparging using of 30-35 Hl water, collected 50Hl wort. This is a delicate step, as the wrong temperature or pH will extract tannins from the chuff (grain husks) as well, resulting in a bitter brew. Total process time of lautering is 140 mins.

Operation of Lauter Tun:

[] After mashing from mash kettle rest for 10-15 min taken.
[] Wort is circulated till the wort got clear, after the bed formation on the false bottom.
[] Transfer the wort to the wort kettle.
[] The gravity is checked.
[] Then sparging of hot water done with temperature about 78°C.
[] Final gravity is checked, and clear wort transferred to wort kettle.
[] The remaining spent grain is removed to the grain kettle. Here near 1100kg spent grain collected per brew and used as cattle feed.

Wort clarity:

Wort separation also aims to achieve consistent wort quality. This can be difficult to measure but typical parameters would be: -

- Wort haze - should be < 50 EBC 10 minutes after the start of run off. This can be measured with an in-line haze meter.
- Suspended solids - no more than 10 to 15 ml as sediment after 2 hours stand in an Imhoff cone.

High hazes in wort can lead to a number of problems either in the brewhouse, or in the process downstream, including

- Run-off problems.
- Excessive volumes of trub / high hazes.
- Flavour problems.
- Fermentation problems (e.g. Due to smothered yeast in extreme circumstances)
- Variable yeast flocculation.

Spent Grains:

The waste husk after the wort has been removed is a valuable by-product because it can be utilized as animal food, typically for cattle. Where used as animal feed, it is necessary to maintain hygienic conditions for the storage and handling of the spent grains, and to ensure no contamination by foreign materials such as oils. Traceability is typically also required. In some breweries, the grain is used as biofuel. To be suitable for this it often has to be further dried using relatively high-pressure belt presses and dried further using the hot exhaust gases from the furnaces.

[] WORT BOILING

Wort Kettle: Wort boiling may be regarded as the turning point in the brewing of beers, many complex reactions take place during wort boiling. As the wort is heated the residual amylases and other enzymes are inactivated which terminates the mashing process and fixes the carbohydrates composition of the wort. At the boiling point, the wort is sterilized, and the micro flora of the malt, hops and other adjuncts used are destroyed wort boiling is a key step in the production of quality beer.

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When the wort has been separated from the malt husk, it is boiled. There are several reasons for doing this:

• To sterilise the wort. Brewing raw materials such as malt, hops and occasionally brewing water itself are infected by micro-organisms. These contaminants are washed into or added to the wort and remain viable, and therefore have to be killed during the brewing process to prevent wort and beer spoilage.

• To stabilise the wort. Above 50 – 80°C, enzyme structure is broken down and the enzymes lose their activity. Thus, a small quantity of enzymes that converted the starch into sugar and the protein into amino acid may not be fully denatured by the final higher temperature stages of mashing, and sparging. Some brewers add external enzymes, such as thermostable beta-glucanase or alpha amylase, intended to help with wort filtration or adjunct degradation (e.g. rice or maize). These enzymes are more heat stable and are active throughout mashing but will be de-activated during wort boiling. It is important that all enzymes are destroyed by boiling, otherwise they would continue working, which would change the profile of the beer.

• To dissolve the bittering resins from the hops and to stabilise them. Hops or hop extracts are added because the bitter resins (alpha acids) dissolve better in hot wort. These alpha acids need to be modified by 'isomerisation' reactions which are heat induced to stabilise the bitterness that is typical of beer flavour.

• To dissolve oils that contribute to hop aroma in the final product, though these generally only remain if the hops are added late, and the oils are not given time to be boiled off.

• To denature and coagulate some of the protein derived from the malt. Protein has the potential to make packaged beer go cloudy as it ages. Its removal at this stage will help protect the beer's stability.

• To develop wort colour and flavour through the action of heat on sugars and amino acids (the chemical reaction between sugars and amino acids is known as the Millard Reaction).

• To increase the strength or concentration of the wort. Wort concentration is a factor in ensuring that the chemical changes described above actually occur. It is also important in the production of strong beers whose original gravity is higher than that of the wort coming from the wort separation system. The amount of water removed during the boil is directly proportional to the rate of evaporation (and hence the amount of energy supplied). The efficiency is affected by the design of the wort kettle, particularly the heating surface area.

• Wort pH continues to fall during wort boiling. The drop in pH is mainly due to the reaction of calcium compounds with phosphates and polypeptides. These form insoluble compounds releasing H+ (hydrogen ions) so reducing the pH.

The kettle design will have a major influence. It has been found that more late-hop character persists in poorly agitated wort.
Wort boiling is a major usage of energy in the brewery and can account for 39% of the brewery's total energy. Steps are taken to make this process as energy efficient as possible by using heat recovery systems

Wort Boiling System :
Kettles are designed to provide a vigorous or 'rolling' boil and to be energy efficient. The main task is to provide sufficient energy input to ensure adequate turbulence and evaporation. A number of different designs have been made to achieve this. Two types of wort boiling systems are present –
• Internal Boiler
• External Boiler

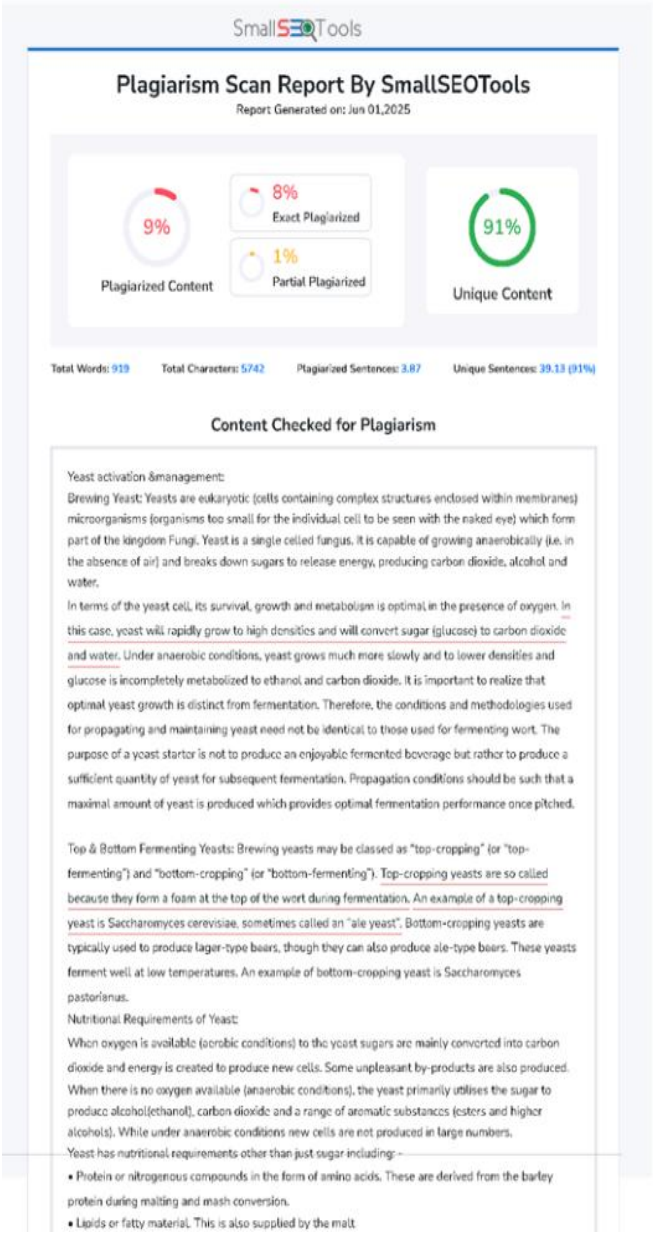
Internal Boiler – In some kettles the heaters were upright and located in the centre to give improve recirculation currents. Some kettles also included base steam coils for preheating the incoming wort, and to minimise dead spots. This was developed further by the introduction of a "chimney" and "spreader plate" giving greatly improved recirculation and a greater surface area for evaporation.
The disadvantage of kettles with internal heating tubes is that the heaters tend to be difficult to clean with conventional CIP due to blind spots and are prone to corrosion. This can result in steam leaks into the boiling wort which are difficult to detect the repair. Wort circulation relies on thermal currents within the kettle and the turbulence over the heating surfaces is limited. This results in high levels of fouling, and will require more frequent cleaning to ensure effective heat transfer in maintained.
The wort kettle which has the capacity of 140 HL consists of an internal boiler.
External Boiler:
Some of the latest designs use an external heater (external wort boiler). The wort is taken out of the kettle and passed through a shell and tube or plate heat exchanger for heating. The heat exchanger may be primed during the pre-boil stage, using a small circulation pump. In some installations when boiling starts, the circulation pump is by-passed. The wort then circulates using the natural "thermosyphon" effect. Circulation starts because hot liquid rises, cools down and therefore sinks again, etc. Incoming wort to the boiler is at 100°C and the outlet wort and superheated vapour from the boiler is around 105°C.
So the hotter liquid rises out of the boiler to the kettle. This saves pumping energy and limits the potential for damage to the "flocs" which would otherwise have to pass through a recirculating pump. The better heat transfer and turbulent conditions improve self-cleaning of the tubes. This allows between 8 and 16 brews (more in most recent designs) to be processed before a clean.

Boiling stabilizes wort by the sterilization and denaturation of enzymes production of bittering compounds, development of colour and protective compounds, flavour balance of hops and malt components.
Hops are dried female flower of hop plant Humulus lupulus. Approximately one quarter pound of hop flower is added per barrel of beer. Before boiling gravity must be tested range ~13. Sugar is added in last 10 mins of 1 hr boiling.

Plagiarized Sources

There are several reasons for doing this:
https://tfc.edu/wp-content/uploads/2023/07/Resume-Tips.pdf

Its removal at this stage will help protect the beer's stability.
https://www.scribd.com/document/469544752/B-G-I-ETHIOPIA-1



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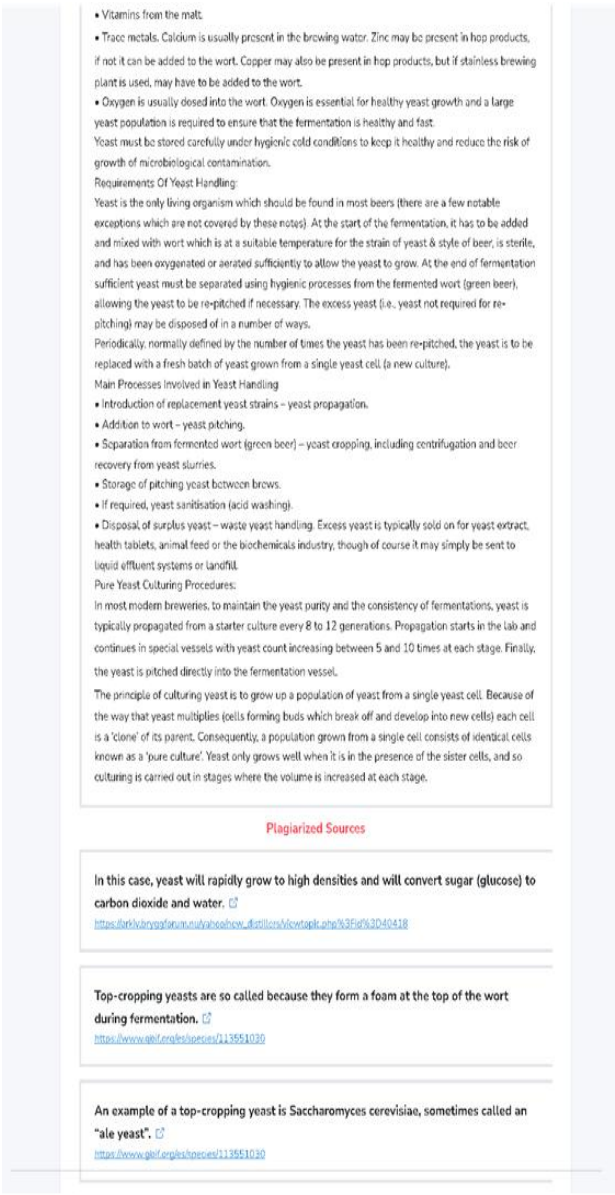
Yeast activation &management:
Brewing Yeast: Yeasts are eukaryotic (cells containing complex structures enclosed within membranes) microorganisms (organisms too small for the individual cell to be seen with the naked eye) which form part of the kingdom Fungi. Yeast is a single celled fungus. It is capable of growing anaerobically (i.e. in the absence of air) and breaks down sugars to release energy, producing carbon dioxide, alcohol and water.
In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water. Under anaerobic conditions, yeast grows much more slowly and to lower densities and glucose is incompletely metabolized to ethanol and carbon dioxide. It is important to realize that optimal yeast growth is distinct from fermentation. Therefore, the conditions and methodologies used for propagating and maintaining yeast need not be identical to those used for fermenting wort. The purpose of a yeast startor is not to produce an enjoyable fermented beverage but rather to produce a sufficient quantity of yeast for subsequent fermentation. Propagation conditions should be such that a maximal amount of yeast is produced which provides optimal fermentation performance once pitched.

Top & Bottom Fermenting Yeasts: Brewing yeasts may be classed as "top-cropping" (or "top-fermenting") and "bottom-cropping" (or "bottom-fermenting"). Top-cropping yeasts are so called because they form a foam at the top of the wort during fermentation. An example of a top-cropping yeast is Saccharomyces cerevisiae, sometimes called an "ale yeast". Bottom-cropping yeasts are typically used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment well at low temperatures. An example of bottom-cropping yeast is Saccharomyces pastorianus.
Nutritional Requirements of Yeast:
When oxygen is available (aerobic conditions) to the yeast sugars are mainly converted into carbon dioxide and energy is created to produce new cells. Some unpleasant by-products are also produced. When there is no oxygen available (anaerobic conditions), the yeast primarily utilises the sugar to produce alcohol(ethanol), carbon dioxide and a range of aromatic substances (esters and higher alcohols). While under anaerobic conditions new cells are not produced in large numbers.
Yeast has nutritional requirements other than just sugar including: -
• Protein or nitrogenous compounds in the form of amino acids. These are derived from the barley protein during malting and mash conversion.
• Lipids or fatty material. This is also supplied by the malt



• Vitamins from the malt.
• Trace metals. Calcium is usually present in the brewing water. Zinc may be present in hop products, if not it can be added to the wort. Copper may also be present in hop products, but if stainless brewing plant is used, may have to be added to the wort.
• Oxygen is usually dosed into the wort. Oxygen is essential for healthy yeast growth and a large yeast population is required to ensure that the fermentation is healthy and fast.
Yeast must be stored carefully under hygienic cold conditions to keep it healthy and reduce the risk of growth of microbiological contamination.
Requirements Of Yeast Handling:
Yeast is the only living organism which should be found in most beers (there are a few notable exceptions which are not covered by these notes). At the start of the fermentation, it has to be added and mixed with wort which is at a suitable temperature for the strain of yeast & style of beer, is sterile, and has been oxygenated or aerated sufficiently to allow the yeast to grow. At the end of fermentation sufficient yeast must be separated using hygienic processes from the fermented wort (green beer), allowing the yeast to be re-pitched if necessary. The excess yeast (i.e., yeast not required for re-pitching) may be disposed of in a number of ways.
Periodically, normally defined by the number of times the yeast has been re-pitched, the yeast is to be replaced with a fresh batch of yeast grown from a single yeast cell (a new culture).
Main Processes Involved in Yeast Handling
• Introduction of replacement yeast strains – yeast propagation.
• Addition to wort – yeast pitching.
• Separation from fermented wort (green beer) – yeast cropping, including centrifugation and beer recovery from yeast slurries.
• Storage of pitching yeast between brews.
• If required, yeast sanitisation (acid washing).
• Disposal of surplus yeast – waste yeast handling. Excess yeast is typically sold on for yeast extract, health tablets, animal feed or the biochemicals industry, though of course it may simply be sent to liquid effluent systems or landfill.
Pure Yeast Culturing Procedures:
In most modern breweries, to maintain the yeast purity and the consistency of fermentations, yeast is typically propagated from a starter culture every 8 to 12 generations. Propagation starts in the lab and continues in special vessels with yeast count increasing between 5 and 10 times at each stage. Finally, the yeast is pitched directly into the fermentation vessel.
The principle of culturing yeast is to grow up a population of yeast from a single yeast cell. Because of the way that yeast multiplies (cells forming buds which break off and develop into new cells) each cell is a 'clone' of its parent. Consequently, a population grown from a single cell consists of identical cells known as a 'pure culture'. Yeast only grows well when it is in the presence of the sister cells, and so culturing is carried out in stages where the volume is increased at each stage.

Plagiarized Sources

In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water.
https://arkiv.brygg.forum.nu/yabbse/new_distillers/viewtopic.php%3Fid%3D40418

Top-cropping yeasts are so called because they form a foam at the top of the wort during fermentation.
https://www.gbif.org/es/species/1135551030

An example of a top-cropping yeast is Saccharomyces cerevisiae, sometimes called an "ale yeast".
https://www.gbif.org/es/species/1135551030

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Yeast Cropping – Reasons & Times:
During the growth phase of fermentation, the yeast population grows approximately five-fold. This additional yeast can be cropped, and the best portions used for pitching into subsequent brews. Yeasts of different strains have different growth rates, and this factor needs to be considered when selecting suitable pitching yeast.
With top fermentations, the time of cropping can be used to select the best yeast for re-pitching. For example, the first and last skimming can be discarded, and the middle crop re-used. Yeast selection like this has enabled some ale brewers to maintain the same yeast strain for many years without culturing.
With bottom fermentations, yeast selection is more difficult, although selection of the most suitable yeast is possible by careful cropping processes. For example, the first purge from the cone can be discarded because it contains trub and a large proportion of dead yeast, and the final purge discarded because it contains some dead cell sand particularly low flocculence yeast, which may be considered unfit for blending back into the main beer stream – with associated loss of revenue and an increase in effluent & disposal costs.
The timing of yeast cropping is therefore to a large extent dependent upon the resistance of the yeast to autolyse when in a settled mass. Cone cooling in itself is of little use as the yeast is a very poor conductor of heat and will not allow the centre of the yeast mass to be cooled.
Yeast Removal Processes
Yeast for re-pitching must be cropped only from fermentations which meet specification for attenuation profile and diacetyl reduction. Wherever possible, microbiological status of fermentations should be available in time to prevent reuse of contaminated or cross-contaminated yeast.
At the end of fermentation in a cylindroconical vessel, the FV is typically cooled to around 4°C and the yeast settles out. The first portion of cropped yeast contains break and dead yeast cells and should go to waste / beer recovery. The next portion can be recovered if required for subsequent re-pitching.
Yeast can be stored as a slurry in beer or pressed to recover the beer, and then either stored "dry", as a cake, or re-slurried with water. Some breweries store the yeast in the cones of the FV and pitch "cone to cone". Yeast can also be recovered from beer on transfer from FV to MV using a centrifuge, though normally the yeast recovered is not used for re-pitching but is sent to waste.

FERMENTATION
The Production of Alcohol & Carbon Dioxide: Most living organisms respire aerobically. They use oxygen to convert sugars to carbon dioxide and water. This releases the energy. Some micro-organisms, including yeast, are able to metabolise anaerobically. They do not need to use oxygen to breakdown carbohydrate. However, the process, known as fermentation is inefficient and instead of carbon dioxide, water and energy, it converts those sugars into alcohol and carbon dioxide plus

considerably less energy. The process starts when yeast is pitched into the wort and finishes when most of the sugar has been converted into alcohol and carbon dioxide.
The overall reaction for fermentation is:
C6 H12 O6 ------- 2 (C2 H5 OH) + 2 (CO2) + Energy
Glucose Ethanol Carbon Dioxide
(sugar) (alcohol)
Fermentation Phases:
1 Lag phase – Nothing happens to the wort specific gravity until the yeast has been pitched in. Yeast growth/reproduction and fermentation do not start immediately. The yeast cells metabolism becomes active following a period of relative inactivity during storage. The length of this phase depends on the type of yeast, the pitching rate, its health, in many cases including the age (number of times it has previously been repitched), and the conditions within the wort. The lag phase is caused by the yeast re-adjusting to the new environment and beginning to absorb and combine the nutrients and oxygen to give them everything required for growth and reproduction. The phase ends with the first cell division.
2.Growth (logarithmic or exponential) phase – The lag phase is followed by a rapid growth phase, starting between 6 and 12 hours after pitching. During the first part of this phase, the rate of cell division continuously increases. The specific gravity drops slowly at first whilst the yeast is growing and dividing. How long the accelerating growth lasts largely depends on the temperature. After about 24 hours the growth rate is constant and at a maximum. The cell numbers can double every 90 to 120 minutes.

Growth is only limited by physical parameters such as temperature and the availability of nutrients such as amino acids and by the amount of oxygen added to the wort. The specific gravity drops rapidly during the fermentation phase. The fermentable sugars are quickly converted into alcohol, large volumes of carbon dioxide are produced, and heat is generated. The pH of the beer also drops during fermentation.
Because specific gravity drops so rapidly and alcohol content increases so rapidly, this phase of the fermentation is often referred to as the Logarithmic (Log) or Exponential phase.

3 Retardation phase- The growth phase is followed by a retardation (late fermentation) phase which is limited by one or more of the following: -
• Available fermentable sugars. • Free nitrogen (amino acids).
• The increase in alcohol level.
• Settling or flocculation of the yeast. And to a certain degree, by lack of recirculation currents keeping the yeast in suspension.
4.Stationary phase – There is a balance between the number of newly formed cells and the cells which die. In the latter stages, sometimes called the declining phase, the rate of cell death exceeds the rate of new cell formation. The final stages of fermentation are slow, and it is where the yeast tends to "mop up" available nutrients that are available. Other biochemical reactions continue, the most important being the removal of diacetyl. Top fermenting yeast may be cropped at this stage. The beer may be reduced in temperature.

Plagiarized Sources

The specific gravity drops rapidly during the fermentation phase.
https://www.clawhammersupply.com/blogs/moonshine-still-blog/how-to-know-when-fermentation-has-finished-advanced%3Fsrsltid%3DAfmBOorAndO-UnkDCBqOSqau1rWYxwk_bhrP3bA1y_ena3QTgmVV5MEVQ

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Factors Affecting the Phases of Fermentations:

- The yeast strain – including flocculation characteristics.
- The age of the yeast – older yeasts tend to produce more sluggish / abnormal fermentations.
- The wort oxygenation level.
- The wort temperature – including the control of the temperature rise.
- The wort composition in terms of nutrients –minerals, vitamins, amino acids.

Factors Affecting The Phases Of Fermentations:

- The pitching rate of yeast.
- The yeast strain – including flocculation characteristics.
- The age of the yeast – older yeasts tend to produce more sluggish / abnormal fermentations.
- The wort oxygenation level.
- The wort temperature – including the control of the temperature rise.
- The wort composition in terms of nutrients minerals, vitamins, amino acids.
- The sugar concentration.

Metabolic Phase During Propagation & Fermentation

Carbohydrate Metabolism for Cell Growth and Energy Generation:

The dominating metabolic pathway of brewer's yeast during beer production is the formation of ethanol by consumption of wort carbohydrates. In general, alcoholic fermentation is an energy generation under anaerobic conditions, whereby glucose is metabolized to ethanol and CO2. The balance of energy is 2ATP/mol glucose.

Acetaldehyde serves as the hydrogen acceptor. In detail, glucose is transferred via glycolysis to pyruvate. The enzyme pyruvate decarboxylase secretes CO2 and acetaldehyde remains. Then alcohol dehydrogenase transforms the aldehyde to ethanol. Under strictly aerobic conditions the same pathways are taken up to pyruvate, but pyruvate is then changed to acetyl-CoA by the pyruvate dehydrogenase. In the following tricarboxylic acid cycle, the acetyl-CoA is used for energy generation. The hydrogen acceptor here is oxygen and 38ATP/mol glucose can be gained.

A very important regulatory phenomenon interacts in this pathway. The Crabtree effect refers to the occurrence of alcoholic fermentation (rather than respiration) of glucose under aerobic conditions because of higher sugar concentrations (Geiger et al., 2004). These sugar concentrations are always found under brewing conditions (e.g., working with wort). Thus, the Crabtree effect occurs constantly during propagation and the start of fermentation and is totally independent of aeration. Despite the

low level of aerobic metabolism, yeast should also grow under brewing conditions. Acetyl-CoA as a precursor for biomass production has to be generated in a different way. Yeast metabolism takes a devious route. The acetyl-CoA is built via the so-called pyruvate dehydrogenase bypass. Here, pyruvate is transferred to acetaldehyde. This is metabolized to acetate and then to acetyl-CoA via two enzymes: (i) acetaldehyde dehydrogenase and (ii) acetyl- CoA synthetase.

Phenolic Compounds:

A special flavour attribute of some German wheat beers is reminiscent of the aroma of cloves. The responsible substances behind this aroma are 4-vinylguaiacol and 4- vinyl phenol. For this type of beer, the flavour of 4-vinylguaiacol and 4-vinylphenol is out most important. The synthesis is regulated by the POF (phenolic off-flavour) gene. This gene can also be found in lager yeasts and even in wild yeasts, but it is only expressed in ale and wild yeast. A contamination with wild yeast results in a phenolic off-flavour in lager beers. For wheat beers, this flavour attribute is desired to a certain degree. It is synthesized by yeast out of the precursor's ferulic acid and coumaric acid. These acids are extracted out of the malt during mashing.

Formation of Sulphur Dioxide:

Sulphur dioxide is generated during primary fermentation by yeast. It is very important as natural antioxidant and enhances flavour stability of the finished beer. The component seems to be formed from intermediates in the pathway that reduces sulphate taken out of the wort. The sulphate enters the yeast by active transport and is reduced enzymatically to sulphite. After another reduction, the resulting sulphide is used for the synthesis of sulphur containing amino acids. When yeast growth ceases, no further sulphur containing amino acids are used and the sulphite is excreted out of the cell. Four phases of sulphur dioxide formation during primary fermentation can be distinguished. Phase 1: No sulphur dioxide excretion because sulphite metabolism is repressed due to a high level of sulphurs containing amino acids. Phase 2: Sulfurdioxide is used for the synthesis of amino acids. Phase 3: Yeast growth ceases, the synthesized sulphur dioxide is excreted. Phase 4: The total metabolism and the sulphur dioxide production come to a standstill. The sulphur dioxide synthesis is directly linked to yeast growth more yeast growth means less SO2. however, reduction of yeast growth to enhance flavour stability has to be assessed carefully.

Fermentation Vessels And Their Control:

The Basic Requirements of Fermenting Vessels

A fermentation vessel has a large number of functions, including those listed below:

- Its prime function is to contain the fermenting wort.
- It must protect the fermenting wort against ingress of foreign micro-organisms which could contaminate the fermentation.
- It must allow fermentation of wort at a controlled temperature by having provision for cooling.
- Sometimes it is considered essential to ferment at a constant pressure to control production of esters and to a lesser extent foam.

When an energy rich substance, such as glucose, is broken down to form a compound with a lower energy content (for example, ethanol), the excess energy (originally derived from photosynthesis when the barley grew) is released. Depending on whether the glucose is respired or fermented the amount of energy released is different. Approximately 25 times more energy is released during the respiration of glucose than during the fermentation of glucose by yeast. During fermentation under production brewing conditions, the amount of heat is approximately 586.6 KJ/kg of fermentable sugar. Wort is usually 70% fermentable. One hl of wort may contain 12 kg (12°P or 1048) of extract. Of this, 8.4 kg is fermented during the primary fermentation. Consequently 586.6 x 8.4 = 4927 kJ are produced by every hl of wort. This heat generated must be removed during fermentation by cooling in order to: -

- Ensure that the fermentation temperature is maintained at the set level which prevents the formation of unwanted flavours.
- Ensure yeast viability.
- Reduce fobbing.

Plagiarized Sources

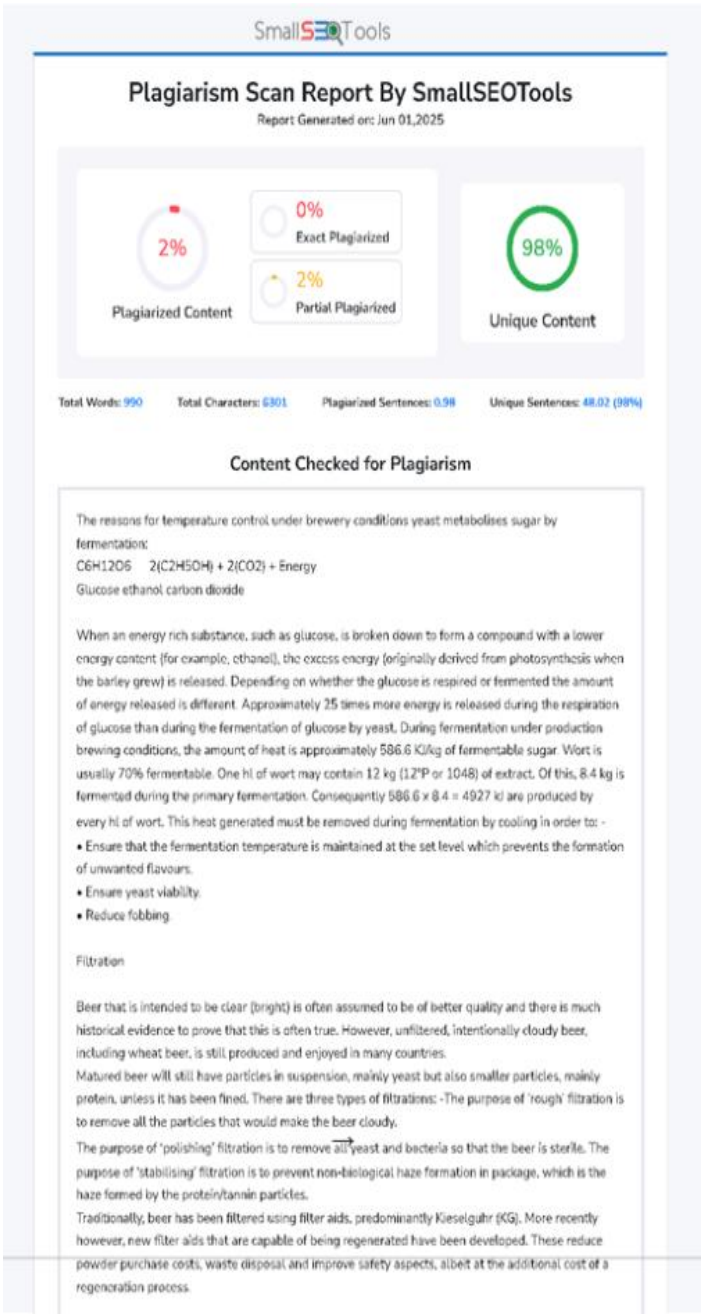
Under strictly aerobic conditions the same pathways are taken up to pyruvate, but pyruvate is then changed to acetyl-CoA by the pyruvate dehydrogenase.

[redacted]

https://homework.study.com/explanation/in-eukaryotes-pyruvate-is-further-metabolized-via-three-main-catabolic-pathways-select-all-correct-statements-i-under-anaerobic-conditions-pyruvate-is-oxidized-leading-to-the-formation-of-acetyl-coa-and-co-2-ii-under-aerobic-conditions-pyruvate-is.html

In the following tricarboxylic acid cycle, the acetyl-CoA is used for energy generation.
https://upload.wikimedia.org/wikipedia/commons/0/0b/Citric_acid_cycle_with_aconitate_2.svg?sa=X

Phase 2: Sulfurdioxide is used for the synthesis of amino acids.
https://www.rexresearch1.com/BrewingLibrary/HandbkBrewingProcessesTech.pdf



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The reasons for temperature control under brewery conditions yeast metabolises sugar by fermentation:

$C_6H_{12}O_6 \rightarrow 2(C_2H_5OH) + 2(CO_2) + Energy$

Glucose ethanol carbon dioxide

When an energy rich substance, such as glucose, is broken down to form a compound with a lower energy content (for example, ethanol), the excess energy (originally derived from photosynthesis when the barley grew) is released. Depending on whether the glucose is respired or fermented the amount of energy released is different. Approximately 25 times more energy is released during the respiration of glucose than during the fermentation of glucose by yeast. During fermentation under production brewing conditions, the amount of heat is approximately 586.6 KJ/kg of fermentable sugar. Wort is usually 70% fermentable. One hl of wort may contain 12 kg (12°P or 1048) of extract. Of this, 8.4 kg is fermented during the primary fermentation. Consequently 586.6 x 8.4 = 4927 kJ are produced by every hl of wort. This heat generated must be removed during fermentation by cooling in order to: -

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- Ensure yeast viability.
- Reduce fobbing.

Filtration

Beer that is intended to be clear (bright) is often assumed to be of better quality and there is much historical evidence to prove that this is often true. However, unfiltered, intentionally cloudy beer, including wheat beer, is still produced and enjoyed in many countries.

Matured beer will still have particles in suspension, mainly yeast but also smaller particles, mainly protein, unless it has been fined. There are three types of filtrations: -The purpose of 'rough' filtration is to remove all the particles that would make the beer cloudy.

The purpose of 'polishing' filtration is to remove all yeast and bacteria so that the beer is sterile. The purpose of 'stabilising' filtration is to prevent non-biological haze formation in package, which is the haze formed by the protein/tannin particles.

Traditionally, beer has been filtered using filter aids, predominantly Kieselguhr (KG). More recently however, new filter aids that are capable of being regenerated have been developed. These reduce powder purchase costs, waste disposal and improve safety aspects, albeit at the additional cost of a regeneration process.

as follows: -

A supporting medium (filter cloth or mesh) is pre-coated with filter aid. The precoat forms a depth filter, but is mainly required to bridge the gaps in the support medium (wire mesh, wedge wire, or cellulose fibre sheet) and prevent the support medium being blinded or allowing filter aid, yeast or proteins to pass through into the final filtered product. After pre-coating is complete, beer is introduced into the filter. To prevent the filter bed blinding with yeast and protein, additional powder, known as body feed is dosed into the beer as it runs into the filter. The yeast and protein particles are adsorbed by the body feed filter powder, gradually building up a layer of powder, yeast and protein until the filter is filled. The filter does not 'blind' because the body feed continually creates a new filter bed.

Diatomaceous earth (DE) or Kieselguhr is made from the skeletons of minute sea plants which have been kilned and milled to various grades of fineness. This is the most porous, rigid and effective filter aid. Kieselguhr can used for both precoat and body feed, depending on the grade (i.e. KG particle size). Kieselguhr is sometimes referred to by trademarked brand names such as Celite.

BEER MATURATION &COLD STORAGE

Introduction: The biochemical, chemical and physical mechanisms involved in flavour changes occurring during maturation are intricate and complex. Maturation includes all transformations between the end of primary fermentation and the final filtration of the beer. Classically fermentation and maturation are considered separate steps in brewing but in practice there is significant overlap. The main difference between traditional maturation systems in ale and lager brewing is that ales are conditioned by warm storage, holding the beer at 12 - 18°C, whilst lagers are conditioned at much lower temperatures (3 - 6°C). There are, of course, always exceptions to this traditional view.

Under warm maturation conditions, residual sugars are rapidly metabolized, and removal of "green" flavours is completed in 1-2 weeks (normally far less) depending on the type of beer, yeast strain, wort composition and primary fermentation conditions.

Maturation of lagers, particularly at low temperature, is significantly dependent upon the length of the lagering (ageing) period, the amount of yeast in suspension and the quantity of fermentable sugar in the young beer or added in the form of actively fermenting wort .Brewery conditioned and filtered beers, i.e. most keg, can and bottle beers, are matured in tanks where flavours improve, the yeast is sedimented out (so aiding filtration), the beer is stabilised to ensure that it stays bright, and oxygen is purged / mopped up by the yeast to very low levels.

dependent, the rate being much higher at higher temperatures and higher yeast concentrations – hence the increase in temperature at the end of fermentation during some lager fermentations.

Because the rate is dependent on the temperature and viable yeast cell concentration, maturation takes place before chilling of the beer and sedimentation of the yeast.

Filtration

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Plagiarized Sources

A supporting medium (filter cloth or mesh) is pre-coated with filter aid.

https://www.sciencedirect.com/topics/engineering/filter-cloth

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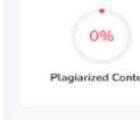

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CO2 in Bottle:
This invention relates to a method of determining the gas content of gas-liquid solutions under pressure, and is more particularly adapted to determine the percentage of co2 in beer, the principal object of this invention is to provide a method of this character which will enable the gas.
Diacetyl Test:
• First 100ml of sample was taken in a beaker.
• Then 3.5ml di sodium hydrogen phosphate was added. Solution was mixed and distilled at 75 .
• 1ml hydroxide ammonium chloride was added to 10ml.20ml distillate was taken sample and set at oil bath till volume 3-5ml.
• Other 10ml distillate was added to it and 1ml disodium hydrogen phosphate was added to it.
• It was set in the spectrophotometer at 220nm to find the absorbance value which gives the equivalent concentration of the diacetyl.
Bottling Hall Check:
Net Volume of Beer:
• Take an empty bottle weight
• Take a weight of filled beer bottle
Weight of beer = (weight of beer bottle-weight of empty bottle)
Volume= (weight of beer bottle -weight of empty bottle)
Specific gravity (1.007)

PU or Pasteurization Unit:
PU value is the duration (in minutes) of the entire pasteurisation process, at a defined temperature.In pasteurization application, especially for drinks, one encounters the term pasteurization units -PU. One PU is defined as exposing a product at 60 for 1 min. Generally, the PU range should be in between 12-30, if less then under pasteurization and if more than over pasteurization.
SENSORY EVALUATION TESTS:
Sensory analysis can provide a qualitative measurement (is beer A sweeter than beer B) as well as a quantitative measurement (on a scale of 1 to 5, beer A is a 4 while beer B is 2 with regards to sweetness). This measurement could be made by trained personnel (a sensory panel) or by consumers, depending on the aim of the study. The aim of the study can further be divided into objective analytical quantity such as flavour intensity or a subjective value such as preference. This basic principle along with asking the right question to the correct sample group at the right time allows breweries to make key decisions about their products and the responses of their consumers to

[] Pathogen testing
[] Indicator organism testing
[] Spoilage organism testing

BEER SPOILAGE MICROORGANISMS - RISKS AND CONSEQUENCES
Due to its low pH value, its high CO2 and alcohol content and the bittering agents it contains, beer generally does not provide good living conditions for most bacteria and is therefore usually not susceptible to contaminations. Nevertheless, some beer spoilage bacteria, like Lactobacillus spp., Pediococcus spp., Pectinatus spp. and Megasphaera spp., have adapted in a way that allows them to grow undisturbed even under these conditions. In most cases, these microorganisms do not pose a health hazard to humans, but can cause off-flavours and thus lead to the loss of entire batches and to significant damage to a company's image. If contamination is suspected or has already occurred, the entire process chain needs to be investigated as quickly as possible. The analysis process can be extremely time-consuming and can even lead to a production stop for a certain period of time. The economic losses in this case are enormous, which is why reliable and fast microbiological quality control is absolutely vital for the brewing industry.

Chemical And Physical Analysis:
• Determination of Colour in Beer
Purpose:- Purpose of the Procedure is to follow appropriate Process for the Determination of
• Colour in Beer.
• Determination of Bitterness in Beer
• Analysis Of Alcohol%, Ae, Re, Og in Beer
• Determination of Haze in Beer
• Determination of PH Test:

It Is Measured by Taking the Sample Beer In A Beaker And Then the Scale Is Dipped in It, The Reading Is Shown by PH Meter Standard Value Of pH 3.5-4.

Beer Analysis as Per Bureau of Indian Standards (BIS) Ethyl alcohol (By Alcolyzer):
Ethanol is an organic chemical compound. It is a simple alcohol with the chemical formula C2H5OH. Ethanol is a volatile, flammable component and the active ingredient in alcoholic drinks. Ethanol is naturally produced by the fermentation of sugars by yeasts. Alternatively, by measuring the density of the starting material and the density of the products using a hydrometer the change in specific gravity during fermentation indicates the alcohol content. Modern digital density meters are based on oscillating U-tube principles. The tube usually a U-shape glass tube, is excited and starts to oscillate at a certain frequency depending on the filled-in sample. Through determination of the corresponding frequency the density of the sample can be calculated.

Presence of Methyl Alcohol:
Sample preparation-
1. KMno4 solution- take 3ml of solid KMNO4, add 15ml ortho phosphoric acid, dissolve in it and finally make the volume 100ml with distilled water, if require filter the solution.
2. Chromo tropic acid- Take 5-gram chromo trophic acid and dissolve it in 100ml of distilled water and filter the solutions.

Test method-
· Take 50ml beer sample add 50ml of water.
· Distilled the solution and collect 2ml in test tube.
· Add 2-3ml of KMNO4 solution in it
· Keep it in deep fridge for 30 minutes.
· Add sodium bi sulphate pinch.

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BRIGHTBEERTANK (BBT)

Tanks must be clean and sterile. They should have smooth internal surfaces. Tanks should be counter pressured with inert CO2 or nitrogen. Beer flow to the tanks should be at the correct flow rate without sharp bends which can promote fobbing. Inlet rate should be smooth to avoid gas breakout in the bottom of the tank. Correct high CO2 and DO levels by purging with oxygen free nitrogen through a sinter at the base of the vessel. Degassing to correct dissolved gas levels will cause some fobbing in the tank. Fob will remain on the vessel walls during emptying and could dry and cause a haze in the next filling if vessel rinsing is ineffective. Sometimes dried bits of collapsed fob fall back into the parent beer and cause similar rejects.
Tanks should be tagged or cooled using jackets. Usually, insulation is sufficient but temperature will rise so a maximum residence period of 2 -3days will be stipulated. Beer warming up will tend to lose dissolved CO2and N2, becomes more prone to micro infection and packaging warm beer leads to fobbing which may allow oxygen pick up and difficulties attaining the required fill levels. It is essential to recheck haze and dissolved gases, CO2, N2 and O2 every shift if the tank is still waiting to be packaged.

Objectives Of Bright Beer Tank:
• Carbonate the beer
• Childproof & stabilize the beer
• Modify & improve beer flavour balance
• Clarify the beer
• Blend & standardize the product

Quality Assurance
MICROBIOLOGICAL TEST
Microbial tests ranging from quantitative and qualitative significantly contribute to the identification of food and outbreak of foodborne diseases. These routine tests can be used to validate primary control methods, such as the Hazard Analysis and Critical Control Point (HACCP) system. Routine microbiological solutions cover the entire production process through to the QA/QC analysis of

Food Safety Measures in Plant:
OPRP-Operational Pre-Requisite Program:
We Have Three OPRP in our Plant. These Are
• Wort Aeration in Brew House.
• Carbonation of Beer in Tank Farm.
• Chlorine-Di-Closing in Water Treatment Plant.

CP-Critical Control Point. All CCPs are in bottling hall.
• EBI: No CCP-checking bottles.
• Filler: No CCP bottle burst at the time of filling.
• Pasteurization: No CCP bottle pasteurization.
(P.R.P) Pre requisite programme:
Prerequisite programmes: It provides the basic environmental and operating conditions essential for the safe production of food.
Examples of prerequisite programmes include:
• Pest Control
• Cleaning and Sanitation (CIP)
• Housekeeping, Personal Hygiene
• Standard Operating Procedure (SOP)
• The Designs and Construction of Food Premises
• Personal Hygiene
• Procurement and Logistics
• Raw and Packing Material Storage
• Finished Goods Storage.

Food Safety Hazards
A food safety hazard is an agent or condition that could potentially cause an adverse human health effect.
Agents are either in or on food and can be either physical, chemical, or biological.

REDUCTION OF OFF-FLAVOUR (DIACETYL) FROM BEER BY YEAST MANAGEMENT SYSTEM
Introduction:
In the theatre of brewing, yeast is viewed as a "supporting actor". But unlike the more commercial leading actors such as water, malt and hops, yeast appears not only once but in many successive productions. Further, with fermentation being the longest chunk of the process, yeast is on stage longer. Finally, and critically, as an agent of change, yeast facilitates the most dramatic event in the process of transformation of wort to beer. Yeast is a single celled microorganism that reproduces by budding. They are biologically classified as fungi and responsible for converting fermentable sugars into alcohol and other by products.
Yeast Life Cycle
The life cycle of yeast is activated from dormancy when it is added (pitched) to the wort. Yeast growth follows four phases, which are somewhat arbitrary because all of the phases may overlap in time:
Lag period
Growth phase
Fermentation phase
Sedimentation phase
Flocculation
The flocculation characteristics of yeast are of great importance. The term "flocculation "refers to the tendency to form clumps of yeast called flocs. The flocs (yeast cells)descend to the bottom in the case of bottom-fermenting yeasts or rise with carbon dioxide bubbles to the surface in the case of top-fermenting yeasts. The flocculation characteristics need to be matched to the type of fermentation vessel used a strongly cropping strain will be ideal for skimming from an open fermenter but unsuitable for acylindro-conical fermenter. A phenomenon shown by brewing yeast is very important for the fermentation performance and following beer production steps flocculation. The ability of yeast

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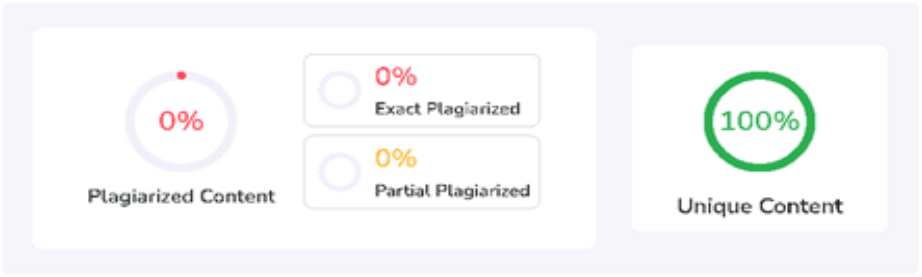

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BEER MATURATION &COLD STORAGE

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Under warm maturation conditions, residual sugars are rapidly metabolized, and removal of "green" flavours is completed in 1-2 weeks (normally far less) depending on the type of beer, yeast strain, wort composition and primary fermentation conditions.
Maturation of lagers, particularly at low temperature, is significantly dependent upon the length of the lagering (ageing) period, the amount of yeast in suspension and the quantity of fermentable sugar in the young beer or added in the form of actively fermenting wort .Brewery conditioned and filtered beers, i.e. most keg, can and bottle beers, are matured in tanks where flavours improve, the yeast is sedimented out (so aiding filtration), the beer is stabilised to ensure that it stays bright, and oxygen is purged / mopped up by the yeast to very low levels.
dependent, the rate being much higher at higher temperatures and higher yeast concentrations – hence the increase in temperature at the end of fermentation during some lager fermentations.
Because the rate is dependent on the temperature and viable yeast cell concentration, maturation takes place before chilling of the beer and sedimentation of the yeast.

Cold storage & stabilization:

The purpose of cold storage improvements in beer flavour are achieved in two stages, warm maturation and cold storage. Yeast must be present for the improvements to occur. Cold storage takes place at 0°C or below and beer flavour continues to improve as the unpleasant compounds are reduced, though the rate of reduction is very much slower than at warm maturation temperatures. The raw materials from which beer is made, especially malt, contain protein material and tannins, which can combine together to form particles large enough to create hazes. This haze is termed non-biological haze because it is not caused by microbiological activity.
Non-biological haze can also be formed after filtration and packaging especially as the beer ages and in the presence of oxygen. A 'chill haze' can be formed if inadequately stabilized beer is stored in a cold place like a refrigerator because the remaining haze particles are less soluble at low temperature.
Other functions of cold storage include: -

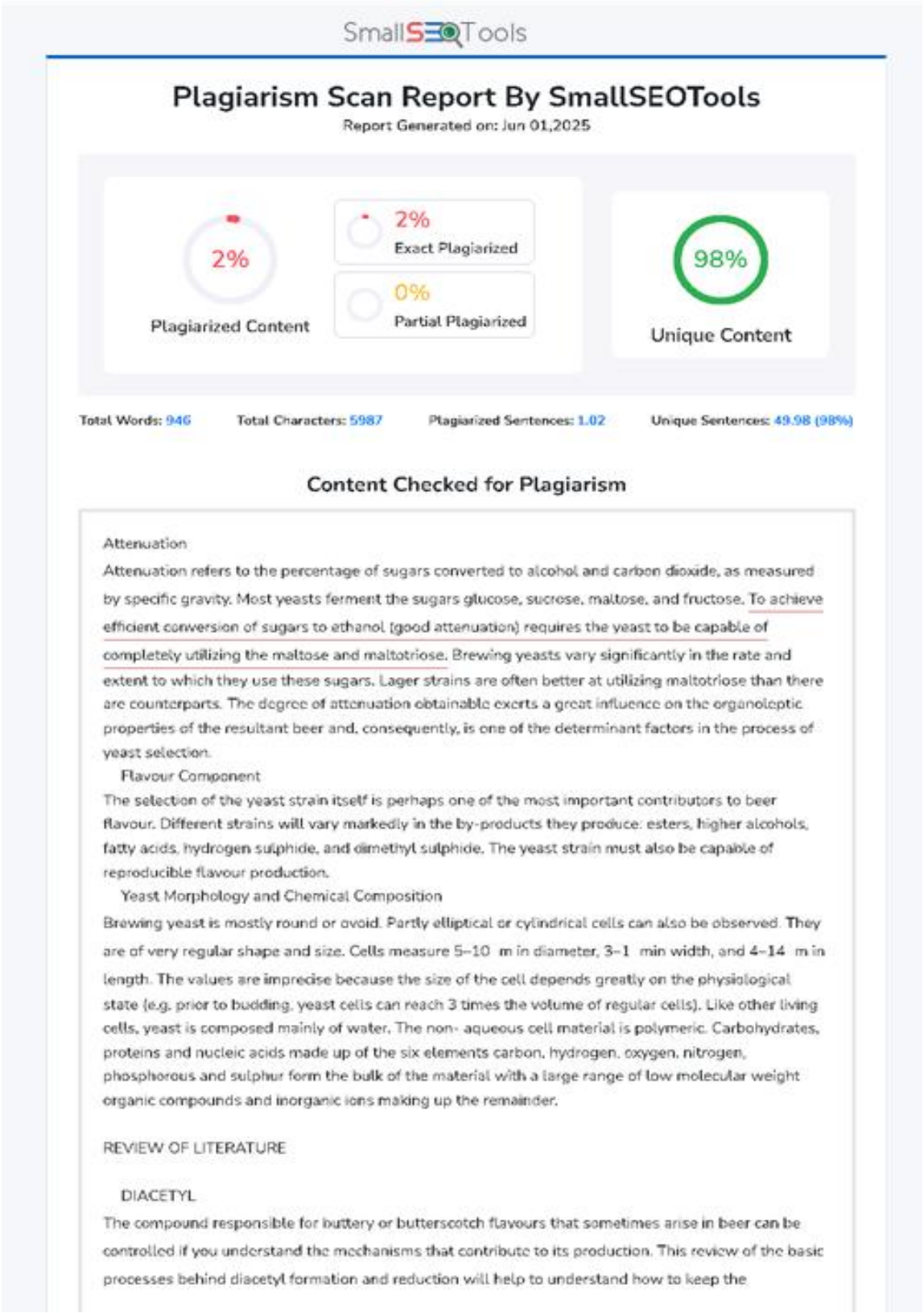
- Oxygen reduction – by allowing residual yeast to mop up oxygen.
- Carbonation - by action of the residual yeast on any fermentable sugars, producing CO_2.
- Stock holding – providing a buffer for periods of high demand.
- Blending to improve consistency of final flavour etc. Ideally to be a routine operation, rather than as a need to bring out of specification beer into specification.
- Process aid reaction time – to allow sufficient time for reactions and settlement to take place.

Typical Times & Temperatures -

Ales typically -1°C for 1 to 4 days, though may be up to a week, particularly for small pack beers. Shorter residence times are most easily obtainable when using beer stabilisers such as silica gels or PVPP. Lagers -1°C for 4 days up to a few weeks, particularly dependent upon the origins of the beer. Traditional style beers are more likely to use longer times than modern recipes. Again, shorter residence times are most easily obtainable when using beer stabilisers such as silica gels or PVPP (where permitted).

General Principles Of Stabilization -

On transfer from FV (or MV) to cold maturation/storage vessel, the beer contains high levels of suspended solids, mainly of yeast and proteins. However, it also contains large amounts of protein and polyphenol precursors. These need to be largely removed before filtration to allow efficient filtration and the required shelf life. The period of cold maturation allows much of the suspended yeast and proteins to settle out. The settlement / removal may be aided by use of centrifuges or processing aids. At 0 to -2°C there is little yeast activity (although lager yeast can grow very slowly at this temperature). The principal changes are physical. Beer haze is formed during cold storage due to the combination of proteins and polyphenols, which either settle out in the storage tank or are removed during filtration. This occurs principally during cold storage and takes around 12 to 36 hours to form. However, the bonds are very fragile and almost instantly break if the beer is warmed up.
Particles, for example yeast and insoluble protein particles, will sediment out as long as they are heavier than the beer. The rate of clarification depends on the size of the particles, how dense the particles are and how far they have to fall. The simplest but lengthiest way of removing the yeast and haze particles remaining in the beer is to allow suspended matter to settle. Stoke's Law tells us the essential features of settling, namely that the rate of sedimentation is increased by: -
- Increasing the diameter of particles.
- Increasing the density of particles.
- Increasing the gravitational force.
- Decreasing the liquid viscosity



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Attenuation

Attenuation refers to the percentage of sugars converted to alcohol and carbon dioxide, as measured by specific gravity. Most yeasts ferment the sugars glucose, sucrose, maltose, and fructose. To achieve efficient conversion of sugars to ethanol (good attenuation) requires the yeast to be capable of completely utilizing the maltose and maltotriose. Brewing yeasts vary significantly in the rate and extent to which they use these sugars. Lager strains are often better at utilizing maltotriose than there are counterparts. The degree of attenuation obtainable exerts a great influence on the organoleptic properties of the resultant beer and, consequently, is one of the determinant factors in the process of yeast selection.

Flavour Component

The selection of the yeast strain itself is perhaps one of the most important contributors to beer flavour. Different strains will vary markedly in the by-products they produce: esters, higher alcohols, fatty acids, hydrogen sulphide, and dimethyl sulphide. The yeast strain must also be capable of reproducible flavour production.

Yeast Morphology and Chemical Composition

Brewing yeast is mostly round or ovoid. Partly elliptical or cylindrical cells can also be observed. They are of very regular shape and size. Cells measure 5–10 m in diameter, 3–1 min width, and 4–14 m in length. The values are imprecise because the size of the cell depends greatly on the physiological state (e.g. prior to budding, yeast cells can reach 3 times the volume of regular cells). Like other living cells, yeast is composed mainly of water. The non- aqueous cell material is polymeric. Carbohydrates, proteins and nucleic acids made up of the six elements carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur form the bulk of the material with a large range of low molecular weight organic compounds and inorganic ions making up the remainder.

REVIEW OF LITERATURE

DIACETYL

The compound responsible for buttery or butterscotch flavours that sometimes arise in beer can be controlled if you understand the mechanisms that contribute to its production. This review of the basic processes behind diacetyl formation and reduction will help to understand how to keep the

and reported as the vicinaldiketones (VDK) content of beer. Brewers' awareness and acceptance of both diacetyl and 2,3-pentanedione have changed dramatically over the past four to five decades. A1952 report, for example, stated that the average diacetyl level of American commercial beer was 0.33 mg/l, more than three times the flavour threshold of 0.10 mg/l.Today the average is near 0.05 mg/l. Some notable exceptions exist. Some stouts can have levels as high as 0.60 mg/l, and a few British pale ales have diacetyl levels near 0.30 mg/l. Diacetyl levels in beers brewed by microbreweries and brewpubs tend to vary considerably, ranging from 0.03 mg/l to more than 1.0 mg/l in cases I have investigated. The presence of diacetyl is usually indicated by a buttery or butterscotch tone. In fresh beer the flavour can be confused with that of caramel malts. Given time it is easy to distinguish the two diacetyl tends to be unstable in most beers and can take on raunchy notes. The flavouring imparted by caramel malts, on the other hand, tends to be stable. The flavour threshold of 2,3-pentanedionels near 1.0 mg/l; it usually imparts flavours that recall honey. This compound can be found well above threshold levels in some Belgian ale, where it is considered a natural flavour constituent for this style. It occurs less often in other beer styles and is regarded as a defect.

Diacetyl Formation

The study of diacetyl and beer began with Pasteur's fundamental work in the 1870s. Using microscopy, Pasteur found that what we know today as lactic acid bacteria were responsible for many off flavours in beer. The term sarcina sickness is used to describe this effect. Apparently, the involvement of diacetyl in sarcina sickness was discovered early, but it was not until 1939 that Shimwell linked this compound with the taste and smell of butter. Even today it is estimated that20% of beer drinkers do not detect the presence of diacetyl even at rather high concentrations.
During the early period, the only known mechanism of Diacetyl formation was bacterial infection caused by unsanitary conditions. Practical brewers believed that some other factor must be involved, because buttery tones occasionally showed up in beer brewed in impeccably clean environments. This work pointed to culture yeast as a major player in both the production and the reduction of diacetyl Inoue and his colleagues at the Kirin Research Laboratory in Japan also made a major contribution by identifying acetoacetic acid as the precursor to diacetyl. This work was followed by a large number of papers in which various facets of diacetyl formation and reduction were studied. Wainwright's excellent 1973 review article contains 149 references. Although a number of factors affect diacetyl formation in beer, the basic middle pathway shown in Figure 1 is the one used for each. The dominant carbon flows down the left most branches, leading to ethanol production. Most beers contain between 30,000 and 50,000 mg/l of ethanol, so a significant amount of pyruvate is processed in this manner. Because the Flavors threshold for diacetyl is 0.10 mg/L as light diversion of the carbon flow to the middle pathway can profoundly affect the flavour of finished beers. Note that this pathway also competes with yeast assimilation and the utilization of the amino acid valine. The practical significance of this is discussed in the section "Wort and Proteins" below.

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To achieve efficient conversion of sugars to ethanol (good attenuation) requires the yeast to be capable of completely utilizing the maltose and maltotriose.
https://www.beer-brewing.com/beer_brewing/beer_brewing_brewers_yeast/yeast_strain_selection.htm

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MATERIALS REQUIRED
UV-Spectrophotometer
Distillation Apparatus (Condenser, Round bottom flask, collector, bent tube, air tight glass wares)
Pipettes
Volumetric flasks
25ml graduated cylinders
Oil bath
Yeast slope
Zink sulphate heptahydrate
Fermenter
Incubator, Autoclave
Slides, Cover Slip Petri plates, Petri plates
Microscope
Bottle turner

Reagents
Hydroxylamine hydrochloride (NH2.OH. HCl) 8% aqueous solution.
Disodium hydrogen phosphate heptahydrates (Na2HPO4) 8.5% aqueous solution.
Saline water (0.85% NaCl).
CuSO4 Solution.
Y.M.C.A (Yeast Malt Agar with Copper Sulphate) Media.
W.L.N(Wallenstein Laboratory Nutrient) Media.

Stock Solution: Dimethyl glyoxime: 0.1349gm of pure dimethyl glyoxime was dissolved in distilled water and the volume was made up to 1000ml by adding distilled water, such that the resulting solution has the concentration equivalent to 100mg per litre of diacetyl.
Working Solution
From the stock solution, 20ml was taken and diluted with distilled water and the volume was made up to 100ml, such that the resulting solution has the concentration equivalent to 20mg/litre of diacetyl.
METHODS
STARTER CULTURE PREPARATION
Yeast Propagation 1. The yeast cells from the slope were removed by using sterile saline water.
2. It was inoculated in two flasks containing 500ml sterile wort each.
3. It was incubated at 25-27°C for 48hrs with constant oxygenation.
4. Then the two 500ml cultures were inoculated in to two flasks containing 5 litres sterile wort and

incubated at 20-22°C for 48hr with constant oxygenation.
5. The two 5 litres culture were inoculated to first yeast propagator containing 1HLof wort heated with 100°C and was cooled to 16-18°C and incubated at 16- 18°C for 24-36hrs.
6. The 1HL cultured was transferred to second yeast propagator containing 10HL wort heated with 100°C and was cooled to 13°C with constant oxygenation.

Yeast Budding Study

A drop of yeast sample was taken over a glass slide. A piece of cover slip was kept over it and viewed fewer than 100X binocular Olympus microscope, for the morphological study.

Fermentation

10HL cultured from yeast propagator was pitched to culture brew of 100HL at 10- 12°C. Online aeration was given during wort collection and the temperature was maintained 10-12°C during fermentation. The fermentation was carried out 7-8 days at temperature 10-12°C till the end gravity and pH 5.5. The fermentation medium composed of amino acids, carbohydrates and proteins.

Wild Yeast Study

Y.M.C.A Media (T.C.M. Media):

24gms of Y.M.C.A was taken in to 1000ml and was autoclaved at 121°C for15mins. 25ml of CuSO4 was added to the media before pouring to the plates. The media was poured to plates and allowed for solidification. After the solidification of media, the sample was poured over it and was kept in incubator for 24hrs.

Sample Measurement:

1. 100ml of undegassed beer was taken in 500ml round bottom flasks.
2. 3.5ml of 8.5% Na2HPO4 was added in to it.
3. Distillation was started with tip of the delivery tube dipping in to 2ml of distilled water.
4. The distillate was collected in 25ml graduated Pyrex cylinder to 18ml and the volume was made up to 20ml by distilled water.
5. 10ml of the solution was transferred to another 25ml graduated Pyrex cylinder and labelled one cylinder for sample and another for blank.
6. 1ml of 8% Na2.OH. HCl was added to the cylinder labelled "sample".
7. Both the cylinders were heated in oil bath at 80°C for 15mins and were cooled in room temperature.
8. 1ml of 8% Na2.OH. HCl was added to the cylinder labelled "blank."
9. 8.5% Na2HPO4 solution was added to both the cylinders. The volume was adjusted to 10ml by distilled water and was mixed thoroughly.
10. The absorbance was taken of cylinder labelled sample vs cylinder labelled blank at 230nm.
11. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.

Study On Reduction of Microbial Contamination During CIP

CIP (Cleaning In Place) plays a significant role in each and every Brewing Industry. It is basically a cleaning process of UTs which comes to play after every batch of fermentation. To reduce microbial load, a sterilant known as Divosan Forte is generally used as final rinse in CIP process.

Study on Antimicrobial Activity of Divosan Forte :

1. Five sample bottles were taken & labelled as Blank,0.1%,0.2% ,0.3%,0.4%.
2. 400ml Soft water which is used for CIP was collected in five numbers of 500mlsample bottle.
3. To increase the microbial load in the water samples some different kinds of microbes such as Acetic Acid Bacteria, Lactobacillus Bacteria, Wild yeast has been added manually.
4. All the bottles were mixed thoroughly by using the bottle turner for 10 minutes.
5. 0.5ml,1.0ml,1.5ml,2.0ml Divosan Forte were added to 0.1%,0.2% ,0.3%,0.4%labelled bottle respectively.
6. Again, all the bottles mixed properly for 10 minutes.
7. Using the Membrane Filter technique, all five samples were filtered through the0.4micron membrane filter using a filter funnel and vacuum system.
8. The filter paper was placed in respective WLN plates and incubated for24hours at 30 degrees Celsius.

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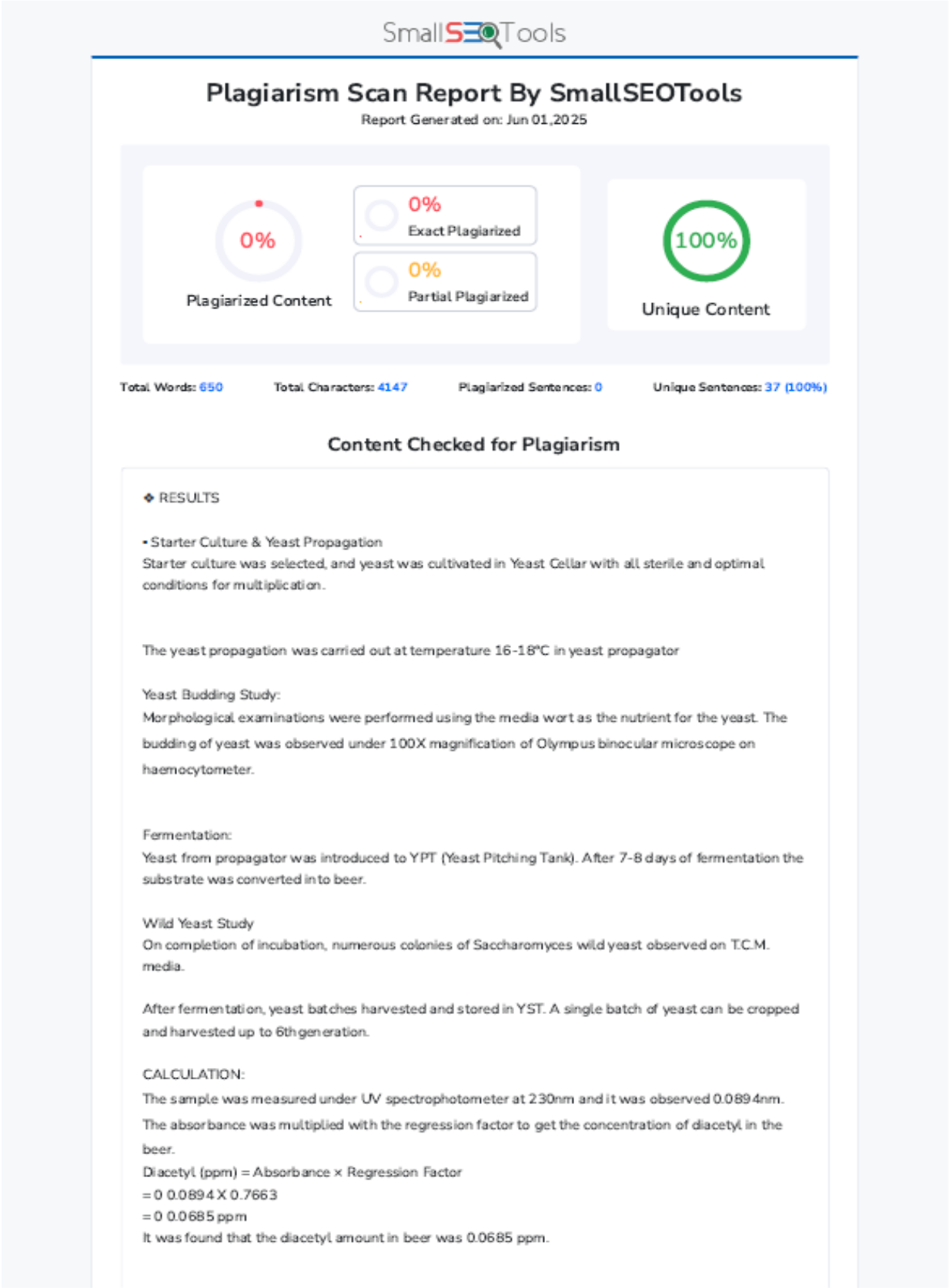
HCl was added to the cylinder labelled "blank."
https://courses.lumenlearning.com/chemistry1labs/chapter/lab-4-pre-lab-assignment

Result:



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❖ RESULTS

• Starter Culture & Yeast Propagation
Starter culture was selected, and yeast was cultivated in Yeast Cellar with all sterile and optimal conditions for multiplication.

The yeast propagation was carried out at temperature 16-18°C in yeast propagator

Yeast Budding Study:
Morphological examinations were performed using the media wort as the nutrient for the yeast. The budding of yeast was observed under 100X magnification of Olympus binocular microscope on haemocytometer.

Fermentation:
Yeast from propagator was introduced to YPT (Yeast Pitching Tank). After 7-8 days of fermentation the substrate was converted into beer.

Wild Yeast Study
On completion of incubation, numerous colonies of Saccharomyces wild yeast observed on T.C.M. media.

After fermentation, yeast batches harvested and stored in YST. A single batch of yeast can be cropped and harvested up to 6th generation.

CALCULATION:
The sample was measured under UV spectrophotometer at 230nm and it was observed 0.0894nm. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.
Diacetyl (ppm) = Absorbance × Regression Factor
= 0 0.0894 X 0.7663
= 0 0.0685 ppm
It was found that the diacetyl amount in beer was 0.0685 ppm.

Discussion:

Diacetyl is naturally produced by all incentive during turmoil and smells like Butterscotch. Diacetyl is naturally produced by all incentive during turmoil and is also "reabsorbed" by incentive cells. Increased diacetyl or diacetyl that is not reabsorbed may be a result of high flocculating incentive, weak or shifted incentive, over or under oxygenating, low turmoil temperatures and weak or short boils. It's generally regarded as an excrescence when detected in lagers. Some spirits, and alkies likewise, desire small quantities in ales.

still, rescuer headache and vomiting will arise after drunk, If diacetyl is more in beer also sickness in the mouth and lingo. The ketone group increases toxin in beer. The entire beer aroma will suppress by VDK. If VDK is more in beer also it can not be consumable.

incentive that's largely flocculent may fall out of suspense before it gets a chance to absorb the diacetyl, using medium flocculation incentive should give the incentive a good chance to absorb diacetyl. Always use high quality incentive and avoid weak or conceivably shifted beaches that may be unable of handling diacetyl duly. Allow incentive to begin original growth with the use of a incentive starter. Sufficient oxygen is to be supplied for incentive growth, but avoid over oxygenating especially after pitching incentive. Allow enough time for incentive to completely raise at applicable temperatures.

Conclusion:

Beer is alcoholic libation consumed each over the world. It reduces the threat of heart complaint, helps the order, packed with nutrition containing vitamin B, VitaminB12, Riboflavin and also rich in silicon which helps to strengthen the bones, reduces threat of diabetes, threat of cancer, and increases brain health if consumed in moderate. However, also similar health problems arise as rescuer headache, puking after drunk, if diacetyl is there in beer. The beer will smell like adulation. The entire beer aroma will suppress by diacetyl.

For Natural enzyme activation in barley, Malting process is done. For high recovery Exogenous enzymes are used that provides that helps maintaining the body of the beer, that improves quality of beer. Because the natural enzymes in malt is not capable to give good recovery and due to use of Adjuncts used in brewery, adjuncts have less enzyme activity. Proper boiling of wort can develop good colour and flavour to the beer

Yeast recovery is done after the fermentation process for recovery of yeast after each generation for reuse of yeast up to sixth generation, so that yeast pitching cost can be reduced. and it is use to six generation only because after sixth generation yeast is not that much viable that it can produce required amount of alcohol in beer processing. If we will use that yeast, it will affect quality of beer like change in flavour